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Feeding Ecology of Capelin (*Mallotus villosus*) in the Estuary and Western Gulf of St. Lawrence and its Multispecies Implications[1]

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VESIN, J.-P., W. C. LEGGETT, AND K. W. ABLE. 1981. Feeding ecology of capelin (*Mallotus villosus*) in the estuary and western Gulf of St. Lawrence and its multispecies implications. Can. J. Fish. Aquat. Sci. 38: 257–267.

The feeding ecology of juvenile (<140 mm TL) and adult (>140 mm TL) capelin (*Mallotus villosus*) in the St. Lawrence River Estuary and western Gulf of St. Lawrence was studied between October 1974 and October 1975. Both juveniles and adults fed almost exclusively on zooplankton. Three prey totally dominated the diet. Copepods were numerically most abundant but euphausiids were dominant in terms of biomass. Appendicularians ranked third in both number and biomass. This differed from the diet of capelin in the Canadian Atlantic only to the extent that amphipods replace appendicularians in the diet in the Atlantic and large copepods are more abundant in the diet there. A shift in diet occurred during growth. Premetamorphic capelin (<75 mm TL) consumed small prey exclusively: invertebrate eggs, cyclopoid copepods, and diatoms predominated. The diets of juvenile (75–139 mm TL) and adult (>140 mm TL) capelin overlapped considerably but a pronounced shift from small copepods to adult euphausiids occurred at ~ 140 mm TL. Early morning and evening feeding peaks occurred between May and August. A single midday feeding peak prevailed in October–November. Feeding of adult capelin was active in the spring prior to spawning but virtually ceased during spawning. Juvenile capelin fed actively throughout spring and summer. The daily ration of zooplankton by capelin was estimated to be 5% of wet body weight in summer May–September and ~ 2.5% in October–November. Year-to-year variations in average daily consumption in excess of 100 000 t have occurred between 1972 and 1978 and average daily consumption has declined by up to 310 000 t during the same interval. This significant year-to-year and long-term release of zooplankton from predation by capelin may be an important regulator of changes in year-class strength in other species of the area, notably arctic cod, *Boreogadus saida* and short-finned squid, *Illex illecebrosus*.

[1]Contribution to the program of GIROQ (Groupe interuniversitaire de recherches océanographiques du Québec).

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D'octobre 1974 à octobre 1975, on a étudié l'écologie alimentaire de capelans (*Mallotus villosus*) juvéniles (<140 mm LT) et adultes (>140 mm LT) dans l'estuaire et le golfe du Saint-Laurent occidental. Les jeunes et les adultes se nourrissent presque exclusivement de zooplancton. Trois proies dominent entièrement le régime alimentaire. Les copépodes sont numériquement les plus abondants, mais les euphausides dominent sous le rapport de la biomasse. Les appendiculaires viennent en troisième lieu, à la fois en nombre et par la biomasse. La seule différence avec le régime des capelans de l'Atlantique canadien est que, dans l'Atlantique, les amphipodes remplacent les appendiculaires et les gros copépodes sont plus abondants dans le régime. Il se produit un glissement dans ce dernier au cours de la croissance. Les capelans d'avant métamorphose (<75 mm LT) consomment exclusivement des petites proies : oeufs d'invertébrés, copépodes cyclopoïdes et diatomée prédominent. Le régime des capelans juvéniles (75–139 mm LT) et celui des adultes (>140 mm LT) chevauchent considérablement, mais il se produit un glissement prononcé des petits copépodes vers des euphausides adultes à environ 140 mm LT. Les pics d'alimentation tôt le matin et le soir se produisent entre mai et août. Un pic unique vers midi prédomine en octobre et novembre. Les capelans adultes se nourrissent activement au printemps avant la ponte, mais l'alimentation cesse pratiquement durant la ponte. Les jeunes capelans se nourrissent activement tout au long du printemps et de l'été. On estime que la ration quotidienne du capelan en zooplancton est de 5% du poids humide en été, de mai à septembre et environ 2,5% en octobre et novembre. Entre 1972 et 1978, il s'est produit des variations annuelles de consommation quotidienne moyenne de l'ordre de plus de 100 000 t métriques. La consommation quotidienne moyenne a diminué de plus de 310 000 t dans la même période. Cette importante libération, d'année en année et à long terme, du zooplancton d'une prédation par le capelan peut être un important facteur de régulation des changements d'abondance des classes d'âge d'autres espèces de la région, en particulier le saida, *Boreogadus saida*, et l'encornet à nageoires courtes, *Illex illecerebrosus*.

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THE capelin, *Mallotus villosus*, is a key element in the marine food web of the North Atlantic Ocean. Bailey et al. (1977b) reported the annual consumption of capelin by minke whales (*Balaenoptera acutorostrata*), fin whales (*Balaenoptera physalus*), harp seals (*Pagophilus groenlandicus*), and Atlantic cod (*Gadus morhua*) in the Northwest Atlantic to be in excess of 30×10^5 metric tons, and listed an additional 23 species of fish, birds, and mammals, known to be major predators of capelin, for which no estimates of consumption rates are available (see also Winters and Carscadden 1978; Minet and Perodou 1978).

In spite of its central role, knowledge of the feeding ecology of capelin and hence the invertebrate base of this important food web remains incomplete principally because previous studies have extended over relatively short time intervals and have differed greatly both in terms of geographical area and size of capelin examined (Jeffers 1931; Templeman 1948; Winters 1970; Kovalyov and Kudrin 1973; Chan and Carscadden 1976).

This paper reports the results of the first comprehensive study of the food and feeding habits of juvenile and adult capelin over a full calendar year. Our object in conducting the study was threefold; to provide new information on the feeding ecology of capelin in the estuary and western Gulf of St. Lawrence; to provide a framework for evaluating the findings of earlier less comprehensive studies; and to provide estimates, for the Canadian north Atlantic, of the average daily consumption of zooplankton by capelin.

Materials and Methods

SAMPLING PLAN

Juvenile and adult capelin were sampled during July, October, and November 1974 and from April through October 1975 during cruises conducted in the area bounded by Ile aux Coudres in the St. Lawrence estuary, Natashquan, on the north shore of the Gulf of St. Lawrence, and the Baie des Chaleurs in the southern Gulf (Fig. 1). All sampling was conducted with a modified Boris midwater trawl (Bergeron 1973) equipped with a headline transducer (Boudreau 1973). Tows were normally of <30 min duration to ensure that discrete schools were sampled. A random sample of 50 fish from each tow was preserved in 10% buffered formalin imme-

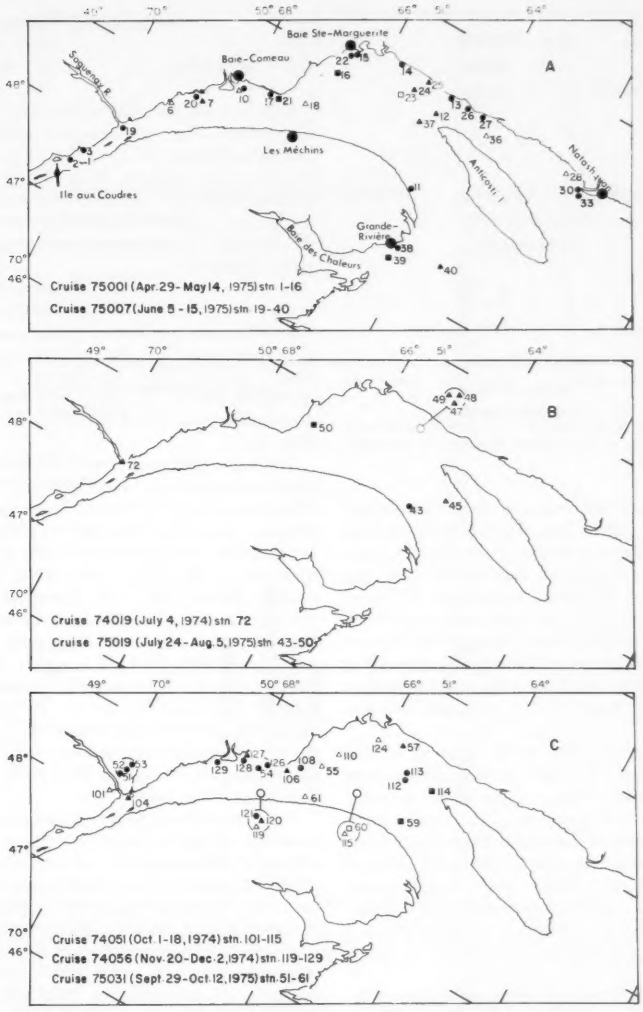
Baie Ste-Marguerite
Baie-Comeau
Saguenay R.
Les Méchins
Ile aux Coudres
Anticosti I.
Natashquan
Grande-Rivière
Baie des Chaleurs
A
Cruise 75001 (Apr. 29 - May 14, 1975) stn. 1-16
Cruise 75007 (June 5 - 15, 1975) stn. 19-40
B
Cruise 74019 (July 4, 1974) stn. 72
Cruise 75019 (July 24 - Aug. 5, 1975) stn. 43-50
C
Cruise 74051 (Oct. 1-18, 1974) stn. 101-115
Cruise 74056 (Nov. 20 - Dec. 2, 1974) stn. 119-129
Cruise 75031 (Sept. 29 - Oct. 12, 1975) stn. 51-61

FIG. 1. Important place names and locations of sampling sites for analyses of feeding intensity and composition of the diet of juvenile (▲) and adult (●) capelin in the estuary and western Gulf of St. Lawrence, 1974–75. (■): juvenile and adult capelin sampled from the same school were analyzed. (□,Δ): capelin were analyzed for feeding intensity but not for diet composition.

diately after capture. Total length (TL mm), wet weight (g), sex, and maturity stage (by gross examination of gonads and secondary sexual characters) were recorded for each fish sampled.

DIET ANALYSIS

When individual collections were composed of fish of uniform size and maturity stage, 15 adult or 10 juvenile fish were selected at random for stomach analysis. When samples from mixed schools contained fish of different sizes and/or maturity stages, we examined a random sample of 15 fish from each category for stomach contents. The number of new prey items identified in any sample when more than 10–15 fish were analyzed was small and did not influence overall estimates of diet composition (Fig. 2).

All food was removed from the stomach, placed on filter paper to remove excess moisture, and weighed to an accuracy

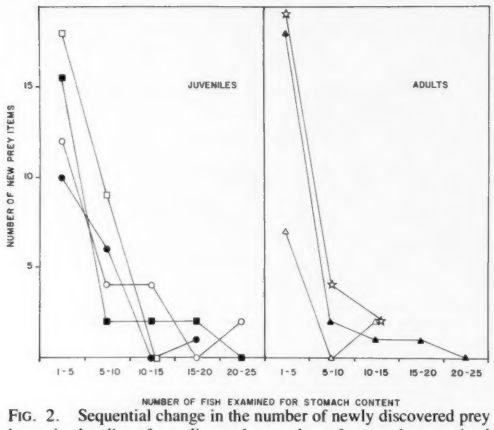

FIG. 2. Sequential change in the number of newly discovered prey items in the diet of capelin as the number of stomachs examined increases.

of 0.1 g. Stomach fullness was expressed as SF = (food wet weight/fish wet weight) · 1000. Daily feeding chronology was determined by first angular transforming individual SF values to stabilize the variance (Sokal and Rohlf 1969; Foltz and Morden 1977) and then calculating means and 95% confidence limits for each sample. Calculated values were then back transformed to the original scale. Data from all stations at which capelin were spawning or had recently spawned at the time of capture were excluded from this analysis because feeding is strongly depressed during spawning (Winters 1970).

The most abundant food items in the diets of all fish were identified to species, all others to order, or to more general categories. Larval stages of the most abundant copepod species were recorded separately. For each stomach examined counts were made of the total number of prey items in each prey category except for appendicularians (Larvacea) which were impossible to separate when they occurred in tangled masses. An index of importance by number ($IN = \sqrt{(\%\text{ composition by number}) \times (\%\text{ occurrence})}$) was calculated for each prey category (Windell 1971; Grabe 1977).

Dry weights were determined for euphausiids, copepods, and appendicularians, the dominant prey of both juvenile and adult capelin, and an index of their importance by dry weight ($IW = \sqrt{\%\text{ dry weight} \times \%\text{ occurrence}}$; Windell 1971) was calculated.

In this paper we define premetamorphic capelin as those fish <75 mm (TL); juveniles as postmetamorphic fish in the length category 75–139 mm (TL), and adults as fish ≥140 mm (TL). This conforms to length ranges reported for the three stages in the study area by Bailey et al. (1977a, b). For the purposes of this study we defined the boundary between the estuary and the western Gulf as a line running from Baie-Comeau to Les Méchins (Fig. 1). This line delimited the presence or absence, in the stomachs of capelin, of *Temora longicornis*, a copepod common in the Gulf (Steven 1974) but absent from the estuary (Côté 1972).

Results and Discussion

Diet Composition

Both juvenile and adult capelin fed almost exclusively on zooplankton (Fig. 3). Copepods were the dominant prey category, *Calanus finmarchicus*, *Temora longicornis*, and *Pseudocalanus minutus* being the principal species consumed. Euphausiids occurred at moderate frequency in the diet but their numerical abundance was low relative to that of the copepods. *Thysanoëssa raschii* dominated the euphausiid group (Table 1).

In spite of their smaller numerical abundance in the diet, the dry weight biomass of euphausiid crustaceans consumed was greater than that of the copepod crustaceans (*IW* 47.1 and 37.36, respectively). Appendicularians (Larvacea) ranked third in terms of their contribution to the biomass of food consumed (*IW* = 7.7). Other prey categories made a minor contribution to the diet both in terms of numbers and biomass (Fig. 3).

The data available from previous studies indicate that copepods, euphausiids, and amphipods are the principal components of the diet of juvenile and adult capelin in the Northwest Atlantic. Chan and Carscadden (1976) reported copepods comprised 50.7%, amphipods 35.9%, and euphausiids 11.1% by weight of the diet of adult capelin sampled from August through January 1973–74. Kovalyov and Kudrin (1973) reported the percent frequency of occurrence (by number) of copepods, euphausiids, and amphipods sampled from March to June 1972 to be 55.4, 44.1, and 31.5%. The major difference between the diets of capelin from the estuary–Gulf and from the Northwest Atlantic was the very low abundance of amphipods (which ranked 20th on the list of 27 prey items) in the diet of capelin from the estuary–Gulf where appendicularians assumed the number three position (after euphausiids and copepods) in the diet.

The reasons for the poor representation of amphipods in the stomachs of capelin sampled in the estuary is unclear. In contrast to their infrequent occurrence in the diet of capelin, amphipods ranked 5th in abundance in 1970 and 4th in 1971 out of 11 holoplankton groups reported for the estuary, occurring in 13.5 and 8.5% of the zooplankton samples taken (Côté 1972). Also conspicuous by their absence from the diet of capelin were fish larvae which ranked 3rd and 2nd in frequency of occurrence in 1970 and 1971, respectively. Copepods, the dominant food item of capelin in the estuary, were also the dominant form of zooplankton in 1971 and 1972, occurring in 97 and 95% of the samples, respectively. Knowledge of the composition and relative abundance of zooplankton in the western Gulf is inadequate to allow meaningful comparisons of diet and prey availability.

Size-Related Differences in Diet

The diet of premetamorphic capelin was dominated by smaller prey items than that of the larger fish. Invertebrate eggs, cyclopoid copepods, and diatoms were major components of the diet and generally increased in importance as the fish increased in length. Nauplii, copepodites, and adults of small copepod species formed the balance of the diet (Fig. 4).

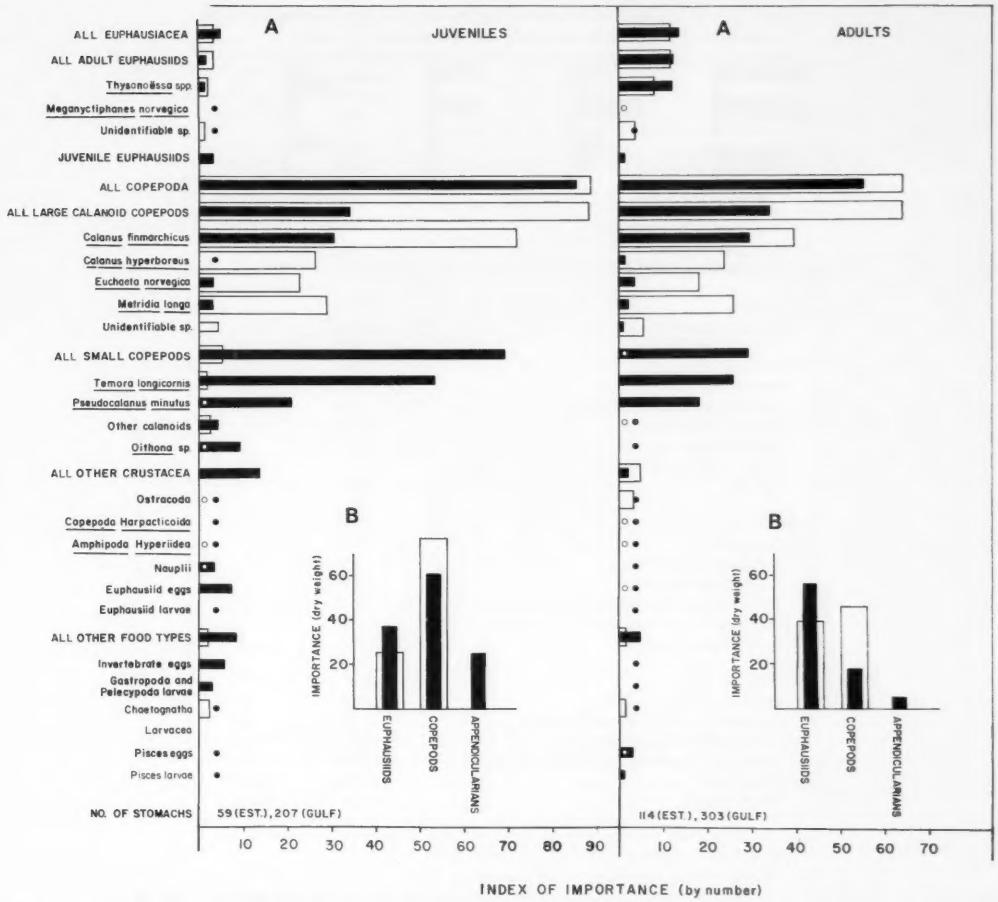

FIG. 3. Index of importance by number (A) and dry weight (B) of components of the diet of juvenile and adult capelin sampled in the estuary (open bars) and western Gulf (solid bars) of St. Lawrence. ○ and ● indicate an index value of 1 for estuary and Gulf, respectively.

The dominant prey of juvenile capelin was copepods, both in numbers and biomass. Euphausiids and appendicularians, while common in the diet, were less important (Fig. 3). Among the residual food items eaten, smaller prey such as invertebrate eggs, nauplii, euphausiid eggs and larvae, and gastropod and pelecypod larvae were more abundant in the stomachs of juveniles than of adults (Fig. 3). Euphausiid eggs formed a major portion of the diet of juvenile capelin in May (Fig. 7). The copepod prey of juvenile capelin was dominated by small species, principally the calanoid species *T. longicornis* and *P. minutus*, and to a lesser extent by cyclopoids of the genus *Oithona*.

In contrast, in the diet of adult capelin, adult euphausiids were significantly more abundant, both in terms of numbers and biomass, and the importance of small copepods was depressed (Fig. 3, 5).

The principal difference in the diet of juvenile and adult capelin was the shift from small copepods to adult euphausiids as fish length increased beyond ~ 140 mm (Fig. 5). We consider it unlikely that seasonal changes in the occurrence of euphausiids were a major factor in their reduced importance in the diet of capelin during the summer and fall (Fig. 7) since they are known to be present in the estuary and western Gulf at that time (Berkes 1976; Sameoto 1976) although this possibility cannot be discounted completely. The apparent absence of small copepods from the diet of juvenile capelin sampled in the estuary may be an artifact resulting from the limited number of juvenile samples from that area since Côté (1972) reported that five of the nine abundant copepods in the estuary were small species. It should be noted, however, that small copepods were also absent from the diet of adult capelin in the estuary which were more adequately sampled. In con-

TABLE 1. Diet composition of capelin from the estuary and the western Gulf of St. Lawrence, 1974–75 (juvenile and adult capelin combined: $N = 683$).

Food item	Frequency of occurrence (%)	% composition (by number)
Euphausiacea	33.4	2.3
Thysanoëssa raschii	17.0	1.2
Thysanoëssa inermis	6.0	0.1
Thysanoëssa sp.	5.3	0.3
Meganyctiphanes norvegica	0.9	0.1
Unidentifiable sp.	5.0	0.1
Euphausiacea juvenile	6.9	0.5
Copepoda	53.2	92.1
Calanus finmarchicus[a]	40.3	27.6
Calanus hyperboreus	13.6	3.0
Euchaeta norvegica	23.1	2.0
Metridia longa[b]	22.1	3.0
Unidentified large Calanoida	5.9	1.7
Temora longicornis	22.3	46.1
Pseudocalanus minutus	16.4	6.8
Other small Calanoida[c]	13.9	0.4
Oithona sp.	10.0	1.5
Other crustaceans	17.7	3.9
Ostracoda	3.4	0.1
Copepoda Harpacticoida	2.2	0.1
Amphipoda Hyperiidea	5.1	0.1
Nauplii (Copepoda, Cirripedia, Euphausiacea)	7.2	0.4
Euphausiacea larvae (calyptopis, furcilia)	1.5	0.1
Euphausiacea eggs	3.2	3.2
Other food types	19.0	1.7
Invertebrate eggs	7.2	0.9
Gastropoda and Pelecypoda larvae	6.4	0.2
Chaetognatha (*Sagitta* sp.)	4.0	0.1
Larvacea (*Oikopleura* sp., *Fritillaria* sp.)	25.0[f]	—
Pisces eggs[d]	2.9	0.4
Pisces larvae[e]	1.8	0.1

[a]No attempt was made to separate the closely related species *C. glacialis* from *C. finmarchicus*. *Calanus glacialis* did, however, occur.

[b]*Metridia lucens* was found in two stomachs.

[c]Includes *Microcalanus* sp., *Eurytemora* spp., *Acartia* spp., *Centropages hamatus*, and an unidentifiable spp.

[d]Almost exclusively capelin.

[e]Almost exclusively capelin. Four redfish larvae (*Sebastes* sp.) were found in one stomach.

[f]Since they were not counted, appendicularians (Larvacea) were not included in the summarizing values of the Other food types category.

trast, small copepods were consumed in large numbers by both juveniles and adults in the western Gulf (Fig. 3).

SPATIAL AND TEMPORAL VARIATION IN DIET

Diel periodicity — Two daily peaks of feeding activity were evident from May to August (Fig. 6). The morning peak, which occurred between 06:00 and 10:00, coincided with the movement of capelin to deeper waters. During midday, when feeding was at a minimum, both juvenile and adult capelin were concentrated into compact schools and were found at their maximum depth (Bailey et al. 1977b). The evening peak, occurring between 16:00 and 22:00, corresponded with the evening vertical migration. This pattern of vertical migration in capelin is essentially the same as that known to occur in many planktonic crustacea (Cushing 1951) and has been documented for euphausiids which are one of the dominant prey groups of capelin in the estuary and Gulf (Berkes 1976). Additional evidence of diel periodicity in feeding during these months is given by our observation that prey items in the stomachs of fish sampled in the 10:00–16:00 period were in a much more advanced stage of digestion than were prey items in stomachs of fish sampled during the moring and evening peaks. The correspondence between the periods of maximum vertical migration and maximum feeding intensity suggest that feeding is most active

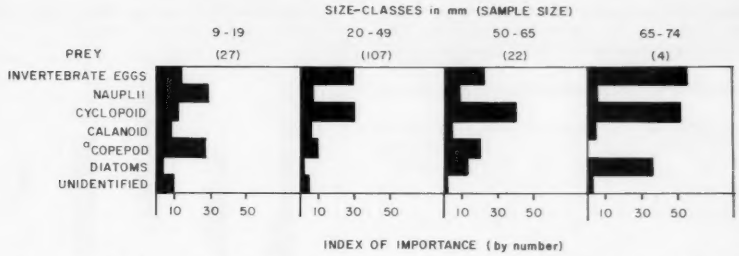

FIG. 4. Index of importance (by number) of components of the diet of premetamorphic capelin. (a) Copepodite stages and adults of small species.

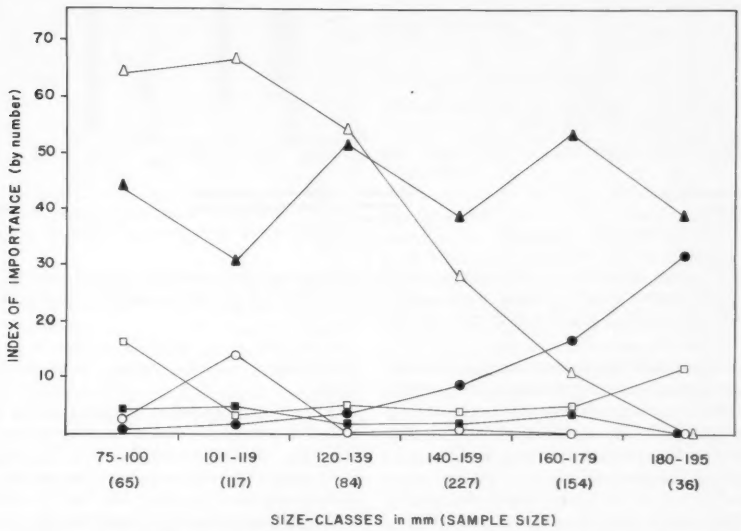

FIG. 5. Index of importance (by number) of prey of varying sizes in the diets of fish of varying sizes. ● adult euphausiids; ▲ large copepods (principally *Calanus finmarchicus*, *C. hyperboreus*, *Euchaeta norvegica*, *Metridia longa*); Δ small copepods (principally *Temora longicornis*, *Pseudocalanus minutus*, *Oithona* sp.); ■ other small crustaceans (nauplii, Amphipoda Hyperiidea, Ostracoda, Copepoda Harpacticoida); ○ euphausiid eggs; □ other food types (invertebrate eggs, Pelecypoda and Gastropoda larvae, Chaetognatha, fish eggs and larvae).

during the transition periods. Similar patterns of intensive feeding during periods of vertical migration were reported by Narver (1970) for juvenile sockeye salmon (*Oncorhynchus nerka*).

A distinctly different feeding chronology appears to prevail in October and November. The intensity of feeding at this time was much more variable, with some samples containing fish having an index of fullness equal to or greater than the maximum observed in the May–August period. Numerous other samples, collected during the same time interval, contained fish whose index of fullness was very low (Fig. 6). A precise interpretation of the daily periodicity of feeding during these months is made difficult by the smaller number of samples available from the dark period. Nonetheless the average index of fullness for 9 night samples was significantly ($P < 0.001$) lower (SF = 5.05; SD 8.42) than the average index for the 18 samples from the light period (SF 11.3; SD 15.79). Similarly, the proportion of empty stomachs in the nighttime samples was higher (night 77.3%; day 59.2%).

These data suggest the presence of a single midday feeding peak in late fall–early winter. The reasons for the apparent shift to a single peak are unknown. One possibility is that the combination of colder (1–2°C), near isothermal waters (Bailey 1977b), which would tend to retard evacuation rates, and the shortened day length, act to merge the summer peaks and reduce the period of active feeding.

Seasonal variation — Capelin exhibited a seasonal varia-

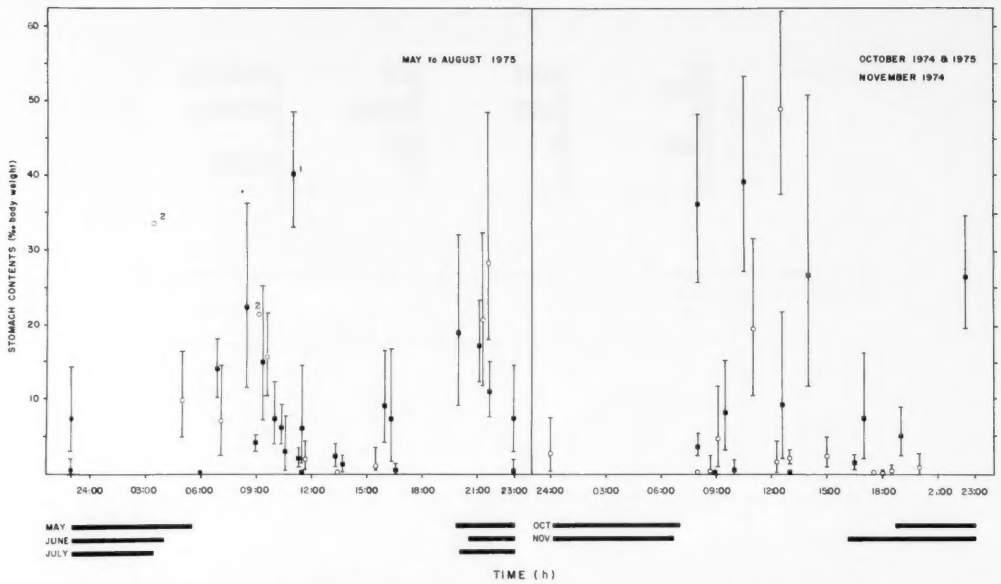

FIG. 6. Diel feeding activity of juvenile (○) and adult (■) capelin from the estuary and western Gulf of St. Lawrence. Mean weights (g) of stomach contents per kilogram of fish per station. Vertical lines above and below means, 95% confidence intervals. Solid bars, hours of darkness. [1]Station 72 (July 1974); [2]Sample based on only four stomachs.

tion in feeding intensity which differed between juveniles and adults and was strongly related to spawning activity. Adult capelin captured prior to spawning in the spring were feeding heavily. For example, in the western Gulf where spawning had not yet begun at the time of the May cruise, the frequency of empty stomachs in the samples was 36% and the mean stomach fullness (SF) was 17.7. In contrast, in the estuary where spawning had already begun (Bailey et al. 1977b) 83% of the stomachs sampled were empty and the feeding intensity was low (SF = 2.7). Many of the stomachs of these spent capelin in these estuary samples contained sand in variable quantities together with small numbers of capelin eggs.

The intensity of feeding by adult capelin sampled in the western Gulf 1 mo later, in June, had declined sharply (empty stomachs 89%; SF = 2.82). Spawning was occurring or had recently terminated throughout the western Gulf at this time. The only exception to this general cessation of feeding by adults during the period of spawning occurred at Baie Ste-Marguerite. There, recently spent females were feeding actively (SF = 14.4). A similar pattern was observed in the southern Gulf at Grande-Rivière. The cessation of feeding by spawning capelin was consistent with the findings of previous studies (Templeman 1948; Prokhorov 1965; Winters 1970).

Very few postspawning capelin were sampled anywhere throughout the study area during the summer. This we attribute to the heavy mortality known to occur after spawning (Templeman 1948; Winters and Campbell 1974; Bailey et al. 1977b; Carscadden and Miller 1979). Where postspawning adult capelin were collected (Stations 43–50, Fig. 1B), they were feeding actively (empty stomachs 14.3 %; SF = 21.1).

As previously noted the feeding intensity of both juvenile and adult capelin in October and November was highly variable (Fig. 6). In general, however, feeding intensity was lower at this time than in the summer months. In 1975, 69% of the stomachs of capelin ≥140 mm TL sampled in October and November were empty and the mean stomach fullness was low (SF = 7.84). Similar-sized capelin sampled in the western Gulf during late fall 1974 had virtually ceased feeding (SF = 1.9, empty stomachs 85%). The decline in intensity of feeding from August through November was similar in magnitude to that reported by Chan and Carscadden (1976).

In contrast to adult capelin, which ceased feeding during the spawning period (estuary April–May; Gulf May–June; Parent and Brunel 1977), juvenile capelin fed actively throughout the spring and summer. The only juvenile capelin sampled in May of 1975 were collected at midday when feeding intensity is at its daily low. Nonetheless, only 12% of these fish had completely empty stomachs indicating that feeding had been active earlier. Juveniles collected in early June, simultaneously with spent females, at several stations in the estuary and Gulf, were feeding actively (SF = 10.9–23.9). During the remainder of the summer and fall the feeding intensity of juvenile capelin was similar to that of the adults (Fig. 6).

In addition to these changes in feeding intensity, some seasonal changes were also observed in the composition of the diet of juvenile and adult capelin. The most significant change

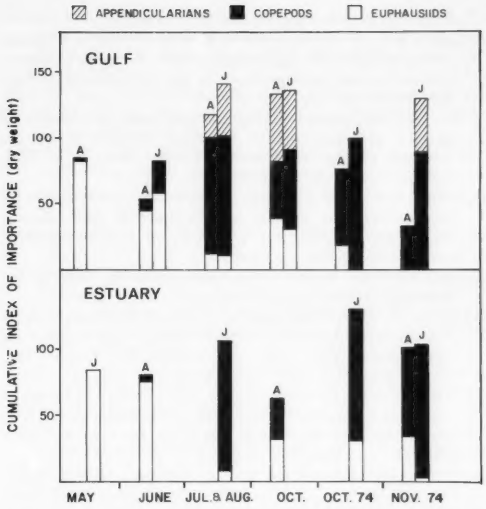

FIG. 7. Seasonal changes in the occurrence of the dominant prey of juvenile (J) and adult (A) capelin in the estuary and western Gulf of St. Lawrence during the fall of 1974 and the summer and fall of 1975.

was the replacement of euphausiids, which dominated the diet of juveniles and adults in May and June, by copepods, principally *Temora longicornis* (Fig. 7). From August through November appendicularians also contributed significantly to the diet of juvenile, and to a lesser extend adult, capelin from the Gulf (Fig. 7). Euphausiid eggs comprised a very large proportion of the diet of juvenile capelin sampled in the Gulf in May ($IN = >90$).

ESTIMATES OF ZOOPLANKTON CONSUMPTION BY CAPELIN

We used the method of Nakashima and Leggett (1978) to estimate daily ration for capelin during the May–August period. Nakashima and Leggett (1978) have shown that this method provides realistic estimates of daily ration provided feeding peaks are discrete and the range of temperatures over the sampling period are small. The former restriction appears to be adequately met. The range of maximum daily temperatures experienced by capelin during their vertical migration increased ~ 13°C between May and August but these higher temperatures were experienced only during their stay near the surface at night. The range of daytime temperatures experienced was ~ 7°C (Bailey et al. 1977b; unpublished data). Errors introduced into estimates of daily ration by the effect of these temperature changes on assimilation rates would be smoothed to some degree by our use of a single representation of feeding peaks for the entire interval.

Daily ration was estimated to be 5% of the wet body weight per day during May–September and 2.5% per day in October–November. This represents a minimum estimate since the Nakashima and Leggett method normally includes the weight of intestinal contents when determining the weight of food consumed whereas we used only the weight of the stomach contents. It nonetheless falls at the midpoint of the range of in situ estimates of daily ration for several species of marine (Clarke 1978) and freshwater (Nakashima and Leggett 1978) fishes.

TABLE 2. Daily summer consumption of zooplankton (principally copepods, euphausiids, and amphipods) by capelin in the Grand Banks and in NE Newfoundland–Labrador regions (metric tons $\times 10^{-3}$).

	1972	1973	1974	1975	1976	1977	1978	1979
Grand Banks (ICNAF 3LNO)[a]	160	115	56	47	41	25	10	No estimate available
NE Nfld.–Labrador (ICNAF 2J+3K)[b]	196	203	191	312	318	208	36	24

[a]Capelin biomass (total minus age 2) estimates from Carscadden and Miller (1979), Table 9.

[b]Capelin biomass (total) estimates from Carscadden and Miller (1980), Table 10.

We estimated the daily consumption of zooplankton by capelin in two areas of the Canadian Atlantic (N.E. Newfoundland — Labrador, ICNAF subareas 2J, 3K; Grand Banks, ICNAF subareas 3LN0) by applying our estimate of daily ration to estimates of total capelin biomass for these regions (Carscadden and Miller 1979, 1980). These data indicate that daily consumption of zooplankton by capelin during the main growing season has ranged from 318 000 to 24 000 metric tons per day between 1972 and 1979 in the 2J, 3K area and from 160 000 to 10 000 metric tons per day between 1972 and 1978 in the 3LN0 area (Table 2). Both areas have experienced a pronounced decline in zooplankton consumption by capelin. In the Grand Banks region in 1978, the last year for which capelin biomass estimates are available, zooplankton consumption by capelin is indicated to have been as much as 150 000 metric tons per day below 1972 levels. In the northeastern Newfoundland–Labrador region daily consumption in 1979 was ~ 194 000 metric tons below 1976 levels. These large-scale changes in zooplankton consumption result, primarily, from the effects of variable year-class success on capelin biomass (Carscadden and Miller 1980; Bakanev 1980). The dramatic increase in the commercial exploitation since 1970 (Winters and Carscadden 1978) may also have contributed, in part, to the general decline.

MULTISPECIES IMPLICATIONS

The pronounced decline in zooplankton consumption by capelin in the Grand Banks region since 1972 and in the northeast Newfoundland–Labrador area since 1976 suggests a surplus biomass of up to 294 000 and 150 000 metric tons of zooplankton per day has become available to other potential predators of zooplankton in these respective areas in recent years. Functional feeding responses (Holling 1965) of capelin to increased food availability could reduce the quantity of zooplankton potentially available to some degree. Nonethe-

less, the magnitude of the changes indicated suggest that recent changes in potential zooplankton availability to other predators could significantly influence growth and/or recruitment in other zooplanktivorous species. Recent evidence suggests that such a response may be occurring in the northeastern Newfoundland–Labrador area.

Maxim (1980) reported the unusual occurrence of large numbers of small arctic cod (*B. saida*) in the catches of the Romanian capelin fleet in ICNAF subdivision 2J during September–October 1979. Massive schools of young arctic cod were also detected by echo-sounder. Large concentrations of young arctic cod have also been recently observed in the area during Canadian research cruises (J. Carscadden, Fisheries and Oceans St. John's, personal communication). The distributions of arctic cod and capelin, while normally distinct, do overlap off Labrador and northeastern Newfoundland (Jangaard 1974; Lear 1979). In this area the diets of the two species are very similar: larval and fry stages (0+) feed primarily on eggs, nauplii, and the copepodite stages of copepods; small and intermediate-size cod 4.2–11.5 cm feed mainly on copepods, and to a lesser degree amphipods and euphausiids; larger cod consume mainly amphipods (Lilly 1980).

The diet and distribution of short-finned squid (*Illex illecebrosus*) also overlap that of capelin. Ennis and Collins (1979) report squid in the Newfoundland area consume significant quantities of zooplankton, principally copepods, euphausiids, amphipods, and chaetognaths. The abundance of short-finned squid, too, has increased dramatically in the Newfoundland region in recent years (G. H. Winters, Fisheries and Oceans, St. John's, personal communication).

The dramatic increase in the abundance of juvenile arctic cod and short-finned squid in the Newfoundland area could thus be a response to the very large reduction in zooplankton consumption by capelin in the region since 1976. No equivalent response has been detected in the Grand Banks area to date. In fact, few, if any, definitive demonstrations of such a functional interaction exist, although numerous examples of correlated changes in species abundance, which are presumed to have a functional basis, have been reported (reviewed in May et al. 1979). The magnitude of the year-to-year changes in capelin biomass in the area, and the strong overlap in the diets of capelin, arctic cod, and short-finned squid suggests that the system may provide an excellent opportunity for the direct study of this important phenomenon.

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Influence of Drilling Muds on the Primary Chemosensory Neurons in Walking Legs of the Lobster, *Homarus americanus*

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DERBY, C. D., AND J. ATEMA. 1981. Influence of drilling muds on the primary chemosensory neurons in walking legs of the lobster, *Homarus americanus*. Can. J. Fish. Aquat. Sci. 38: 268–274.

The effects of whole drilling muds on the normal activity of walking leg chemosensory neurons of the lobster, *Homarus americanus*, were examined using extracellular neurophysiological recording techniques. Exposure of legs for 3–5 min to 10 mg/L drilling mud suspended in seawater altered responses to food odors of 29% of the chemoreceptors examined (data pooled for the two drilling muds tested); similar exposure to 100 mg/L drilling mud resulted in interference with 44% of all receptors studied. The effects of both of these concentrations are statistically significant, although they are not different from each other. Interference was usually manifested as a marked reduction in the number of action potentials in a response. In one preparation, the exposure to drilling mud caused a change in the temporal pattern of the spikes without affecting the total number of spikes. Other chemosensory neurons were excited by 10 mg/L drilling mud itself. However, not all chemoreceptors are inhibited by these drilling muds since responses to feeding stimuli were recorded from the legs of lobsters that had been exposed to drilling mud for 4–8 d before the neurophysiological experiments.

Antennular and leg chemoreceptors are important in eliciting normal feeding behavior in lobsters. Although behavioral assays have demonstrated that feeding behavior is altered following exposure to drilling muds and petroleum fractions, there is no conclusive proof for a causal relationship between chemoreceptor interference and behavioral deficits. The two techniques complement each other as pollution detection assays, perhaps reflecting a common interference mechanism.

Key words: chemoreception, drilling mud, feeding behavior, lobster, pollution, neurophysiology

DERBY, C. D., AND J. ATEMA. 1981. Influence of drilling muds on the primary chemosensory neurons in walking legs of the lobster, *Homarus americanus*. Can. J. Fish. Aquat. Sci. 38: 268–274.

Des méthodes d'enregistrement neurophysiologiques extracellulaires ont servi à étudier les effets des vases de forage sur l'activité normale des neurons chimiosensoriels des pattes ambulatoires du homard, *Homarus americanus*. Une exposition des pattes durant 3–5 min à 10 mg/L de vase de forage en suspension dans de l'eau de mer influe sur les réponses à des odeurs de nourriture de 29% des chimiorécepteurs examinés (groupage des données des deux vases de forage testées); une semblable exposition à 100 mg/L de vase de forage produit une interférence dans 44% de tous les récepteurs étudiés. Bien que les effets de l'une et l'autre de ces concentrations ne diffèrent pas entre eux, ils sont significatifs à l'analyse statistique. L'interférence se manifeste ordinairement par une diminution marquée du nombre des potentiels d'action dans une réponse. Dans une préparation, l'exposition à la vase de forage produit un changement dans le schéma temporel des crêtes, sans agir sur le nombre total de ces dernières. D'autres neurons chimiosensoriels sont excités par 10 mg/L de vase de forage seule. Cependant, ce ne sont pas tous les chimiorécepteurs qui sont influencés par ces vases de forage puisqu'on a enregistré des réponses à des stimuli alimentaires sur des pattes de homards qui avaient été exposés à de la vase de forage pendant 4–8 d avant les essais neurophysiologiques.

Un rôle important des chimiorécepteurs des antennules et des pattes est de provoquer le comportement alimentaire normal chez les homards. Bien que des expériences sur le compor-

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tement aient démontré que le comportement alimentaire change après exposition aux vases de forage et aux fractions d'hydrocarbures, il n'y a pas de preuve définitive d'une relation causale entre l'interférence subie par les chimiorécepteurs et un comportement aberrant. Les deux types d'expériences se complètent comme méthodes analytiques de détection de la pollution, signe possible d'un mécanisme d'interférence commun.

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DRILLING muds (drilling fluids) are a group of potentially hazardous pollutants. Drilling muds are mixtures of clays, water, and chemical additives used during oil and gas drilling operations to lubricate the bit, remove drilled cuttings, and equalize hydrostatic pressure. The drilling muds are continually released into the surrounding waters during the drilling operation and, in addition, are released in bulk upon completion of the drilling operation. The compositions of drilling muds vary, but they often contain suspended drilled solids, weighting agents (barite, bentonite clays), viscosifiers (chromium lignosulfonates), fluid-loss reducers, flocculants, dispersants, detergents, surfactants, defoamers, emulsifiers, lubricants (processed hydrocarbons), bactericides (sodium pentachlorophenate), and alkaline compounds (Land 1974; Wright 1975). Whole drilling muds or individual components of drilling muds have caused sublethal and lethal effects on marine organisms (e.g. Thompson and Bright 1977; Tagatz et al. 1978; Rao et al. 1979; Conklin et al. 1980), including lobsters (S. Oleszko-Szuts, J. Atema, E. Karnofsky, and R. Elgin, 1979, unpublished data).

Chemoreception is probably the dominant sensory modality with which lobsters and most other benthic organisms relate to their environment, using it to obtain food (Ameyaw-Akumfi 1977; Hirtle and Mann 1978; Reeder and Ache 1980) and mates (McLeese 1970; Atema and Engstrom 1971; Gleeson 1980). Various chemical pollutants, including such potential components of drilling muds as surfactants, detergents, dispersants, and petroleum, are known to affect adversely chemically mediated behaviors of many marine organisms, including bacteria, invertebrates, and vertebrates. Petroleum inhibits bacterial chemotaxis (Walsh and Mitchell 1973). Such chemically elicited behaviors of crustaceans as pheromone detection and food detection, location, and consumption can be interfered with by different pollutants (Swedmark et al. 1971; Takahashi and Kittredge 1973; Milovidova 1974; Evans et al. 1977; Lyes 1979). In addition, the feeding behavior of both larval (Wells and Sprague 1976) and adult (Atema and Stein 1974; Atema 1976; Atema et al. 1979b) lobsters can be deleteriously affected by the water-soluble and water-accommodated fractions of petroleum.

It has been assumed in many of these studies that interference takes place at the primary sensory receptors themselves; however, this assumption has rarely been examined. The structure and function of chemoreceptors of some fish may be impaired by detergents and certain ionic surfactants (Bardach et al. 1965; Sutterlin et al. 1971). Neurophysiological experiments with chemoreceptors in lateral flagella of antennules of *H. americanus* indicate that 1−10 mg/L No. 2 fuel oil added to a mussel extract causes nerve-firing patterns different from those elicited by the mussel extract alone (Atema et al. 1979b).

Direct pollutant effects on the chemoreception process can be measured by a neurophysiological test such as is described here. Chemoreceptors in the legs are of particular interest since they are heavily exposed when the pollutant is part of the substrate, as is the case with drilling muds. The experiments presented here demonstrate that a short-term exposure of 10- or 100-mg/L drilling mud can interfere with the normal physiological activity of the primary chemosensory cells in the walking legs of the lobster, *H. americanus*.

Materials and Methods

The effects of short-term exposure of whole drilling muds (DM) on the physiology of primary sensory neurons were examined by recording neurophysiological responses from chemoreceptors in the walking legs of lobsters. Two types of drilling muds were tested: "DM-2" (density = 1.14 g/mL, pH = 11) and "DM-3" (density = 1.51 g/mL, pH = 11), both collected from wells in Mobile Bay, Gulf of Mexico, and supplied by Dr Norman L. Richards, U.S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, Florida.

Legs from two groups of lobsters were tested: "clean" lobsters and "DM-treated" lobsters. All animals were individually held in 100-L aquaria with a flow-through seawater system and a 5-cm layer of sand covering the bottom. DM-treated lobsters received a single introduction of 100- or 1000-mL DM (resulting in a 1- to 10-mm layer of DM on top of the sand) 4−8 d before the neurophysiological experiments were performed. They were treated in the aquaria and tested in the neurophysiological experiments with the same type of DM. Clean lobsters did not receive this 4- to 8-d DM treatment.

Neurophysiological experiments were performed on the walking legs of these lobsters. After a leg was removed from a lobster, the exoskeleton of all but the two most distal segments (dactylus and propodus) was carefully dissected away, exposing the sensory nerve bundle. These two remaining intact segments were inserted through a rubber dam in a Plexiglas chamber (modified from the design for antennules described by Atema et al. 1979b) so that this leg section was isolated from the exposed nerve. The nerve was bathed in *Homarus* Ringer's solution, and a constant stream of filtered seawater (10 mL/min) flowed over the distal leg region. Chemical stimuli (50 μL) were injected into this flow, and neural responses from chemoreceptors that had been separated from the main sensory nerve bundles were recorded extracellularly with a platinum hook electrode. Two chemical stimuli known to activate lobster chemoreceptors strongly

TABLE 1. Protocol for analysis of short-term effects of drilling muds.

Background flow[a]
1. FSW
2. 10 mg/L DM
3. FSW
4. 10 mg/L DM
5. FSW
6. 100 mg/L DM
7. FSW
8. 100 mg/L DM
9. FSW

[a]The legs were exposed to each background flow for 3–5 min before the responses to at least two presentations of each of the following were recorded: L-glutamate, mussel extract, and filtered seawater (FSW). For DM-2, backgrounds 1–5 were tested; for DM-3, backgrounds 1–9 were tested.

(Derby and Atema 1978) were used: mussel (*Mytilus edulis*) extract (100 mg tissue/L filtered seawater) and 10^{-5} mol/L L-glutamate. The introduced chemical stimuli were diluted in the chamber by a factor of 30 when they reached the leg receptors, as determined independently by spectrophotometric measurements following dye injection and by measuring conductance changes following injection of a salt solution. The reported concentrations of introduced stimuli include this dilution factor.

In one set of experiments, responses to an addition of 50 μL of 10 mg/L DM were recorded from chemoreceptors of clean and DM-treated lobsters to determine if DM itself can stimulate chemoreceptors in the legs. In a second set of experiments, short-term effects of DM were tested by monitoring the chemoreceptor responses to the following stimuli: L-glutamate, mussel extract, and filtered seawater (control stimulus). These stimuli were presented in three different background flows: filtered seawater (FSW), 10 mg/L DM in FSW, or 100 mg/L DM in FSW. The protocol used is described in Table 1. A loss of response to food odors following the exposure to DM could be due either to an effect of DM on the neurons or to a recording problem with the neurophysiological preparation (e.g. loss of neuron-to-electrode contact). Therefore, to ensure that the effect was due only to DM, the entire set of recordings was voided if the response to the food odor was not retrieved following a period of rinsing with FSW. Recordings were made from only one nerve bundle per leg to avoid effects of previous exposure on subsequent tests. For DM-2, responses were recorded from seven legs of five clean lobsters and from seven legs of six DM-treated lobsters. For DM-3, responses were recorded from 11 legs of eight clean lobsters and 13 legs of six DM-treated lobsters. Neural activity was amplified on standard electrophysiological equipment and stored on magnetic tape for subsequent analysis.

Results

Introduction of 50 μL of a 10-mg/L drilling mud (DM) stimulus into the FSW background flow elicited responses (e.g. Fig. 1), whereas control stimuli (FSW) did not, demonstrating that DM itself can be sensed by chemoreceptors. No

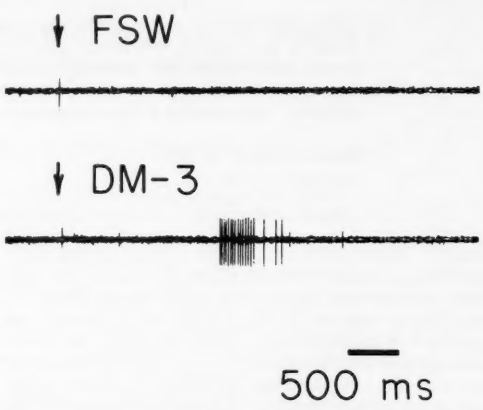

FIG. 1. Detection of 10 mg/L DM-3 stimulus by chemoreceptor on leg of DM-treated lobster. Arrow indicates stimulus introduction.

chemoreceptors of clean lobsters responded to DM-2, but 17% responded to DM-3; 20% of the chemoreceptors of DM-treated lobsters responded to DM-2, while 46% responded to DM-3 (Table 2). The difference between responses to 10 mg/L DM stimuli and to filtered seawater stimuli is significant ($P < 0.05$, sign test; data pooled for DM-2 and DM-3 and for clean and DM-treated lobsters).

Short-term exposure to DM-2 and DM-3 caused interference of the responses to food odors. Interference is defined as a clear reduction in the total number of action potentials (spikes) in the response to either L-glutamate or mussel extract during exposure to drilling mud as compared to the response before and after exposure. Representative examples of such interference caused by DM are illustrated in Fig. 2 and 3. In one case, rather than reducing the number of spikes elicited by the mussel extract stimulus, the exposure to DM caused a clear and reproducible modification of the temporal pattern of the spikes (Fig. 4). This change in response pattern is considered to be an interference effect caused by DM. Figure 4 is an example of the reproducibility of responses to repeated introductions of the same stimulus. Short-term exposure to 10 mg/L DM-2 caused interference in 57% of the clean lobsters (Fig. 2), but not in 4- to 8-d DM-treated lobsters. Short-term exposure to 10 mg/L DM-3 caused interference in 18% of the clean lobsters and in 44% of the DM-treated lobsters (Fig. 4). Exposure to 100 mg/L DM-3 caused interference in 50% of the clean lobsters (Fig. 3) and 38% of the DM-treated lobsters (Fig. 4). The results are summarized in Table 2. The sign test (Siegel 1956) demonstrates that the interference effects of both 10- and 100-mg/L DM-3 are statistically significant (both $P < 0.05$; data pooled for clean and DM-treated lobsters), and that while the effect of 10 mg/L DM-2 does not reach this probability level, it does show a similar trend ($P = 0.06$). The Fisher exact probability test finds no significant difference ($P < 0.05$) between the effects of 10- and 100-mg/L DM.

Discussion

Examination of the activity of chemosensory neurons is an

TABLE 2. Effect of drilling muds on chemoreceptors: (A) excitation and (B) interference of responses to food odors.

Treatment of lobsters previous to neurophysiological test[a]	Type of DM	Excitation	Interference of responses to food odors	
			10 mg/L DM	100 mg/L DM
Clean lobsters	DM-2	0/5[b] (0.0%)	4/7[c] (57.1%)	—
	DM-3	1/6 (16.7%)	2/11 (18.2%)	4/8 (50.0%)
DM-treated lobsters	DM-2	1/5 (20.0%)	0/7 (0.0%)	—
	DM-3	6/13 (46.1%)	4/9 (44.4%)	3/8 (37.5%)

[a]See Materials and Methods for explanation of treatments.
[b]The ratio of the number of chemoreceptors responding to a 10-mg/L DM stimulus and the number of chemoreceptors tested.
[c]The ratio of the number of chemoreceptors in which the response to the food odor was clearly altered by DM and the number of chemoreceptors tested. Dashes signify that this DM concentration was not tested.

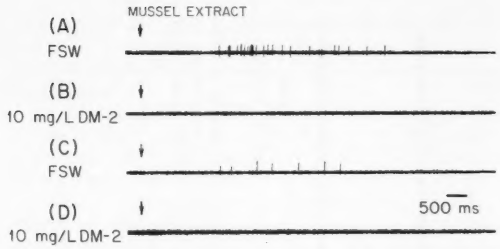

FIG. 2. Complete inhibition of clean lobster leg chemoreceptor response to mussel extract stimulus by DM-2. Mussel extract was introduced into the following background flows: (A) FSW; (B) 10 mg/L DM; (C) FSW; (D) 10 mg/L DM. Arrows indicate stimulus introduction.

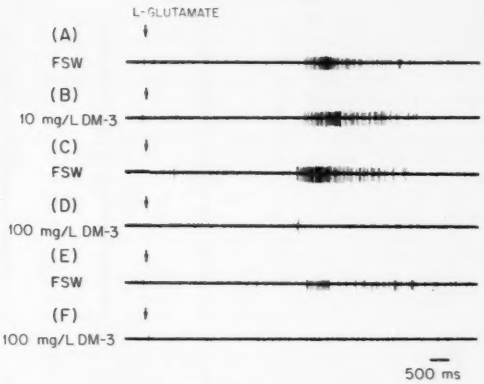

FIG. 3. Inhibition of clean lobster leg chemoreceptor response to 10^{-5} mol/L L-glutamate stimulus by DM-3. L-Glutamate was introduced into the following background flows: (A) FSW; (B) 10 mg/L DM; (C) FSW; (D) 100 mg/L DM; (E) FSW; (F) 100 mg/L DM. Arrows indicate stimulus introduction.

effective method of monitoring the influence of drilling muds on chemoreception. Reproducible results of drilling mud-induced changes of the responses of chemoreceptors to feeding stimuli have been obtained: a 3–5-min exposure to 10- or 100-mg/L whole used drilling mud altered the normal activity of 33 and 50%, respectively, of the leg chemoreceptors of all clean lobsters examined (Fig. 2–4; Table 2). In addition, 25 and 37.5% of the chemoreceptors in legs of long-term treated (4–8 d) lobsters were inhibited by 10- and 100-mg/L drilling muds, respectively. However, normally functioning receptors were found in these drilling mud-treated lobsters, indicating that not all chemoreceptors are altered by such long-term drilling mud treatments. It has also been demonstrated that there are chemosensory neurons on the walking legs that respond to drilling mud itself. Drilling mud excited chemoreceptors in 28% of all nerve bundles that responded to either a mussel extract or L-glutamate (Fig. 1; Table 2). The possible behavioral consequences of these sensory physiological effects are discussed later.

It is not known which components of drilling muds directly excite certain chemoreceptors or which components inhibit the responses of other chemoreceptors to feeding stimuli. Nor has the cellular mechanism of this interference been determined in this study. Structural damage of chemoreceptors following exposure to pollutants can occur in some aquatic species (Bardach et al. 1965), although there is no evidence that oil causes changes in the gross cellular morphology of antennular chemoreceptors of lobsters (Atema and Stein 1974). The interference could possibly result from a physical blockage of the receptor surfaces so that odorant molecules cannot reach the receptor sites on the chemoreceptor membrane surface. A more likely possibility, however, is that some components of the drilling muds are complexing with the membrane lipids (Hodgson and Steinhardt 1967; Kaissling 1977) and/or proteins (Manwell and Baker 1967; Boldacre 1968), thus disrupting the transduction process (Poynder 1974).

Behavioral consequences of these effects of drilling muds on chemoreceptor function are not known, but parallel behavioral experiments indicate that adult lobsters held in a flow-through system show suppressed or abnormal feeding behavior when exposed to a 1-mm layer of certain types of drilling muds (Oleszko-Szuts et al., 1979, unpublished data). These results are in agreement with oil-induced changes in chemoreception and feeding behavior of lobsters (Atema 1976; Atema et al. 1979b). Numerous behavioral studies have demonstrated that other decapod crustaceans can also detect chemical pollutants and may respond with avoidance, attrac-

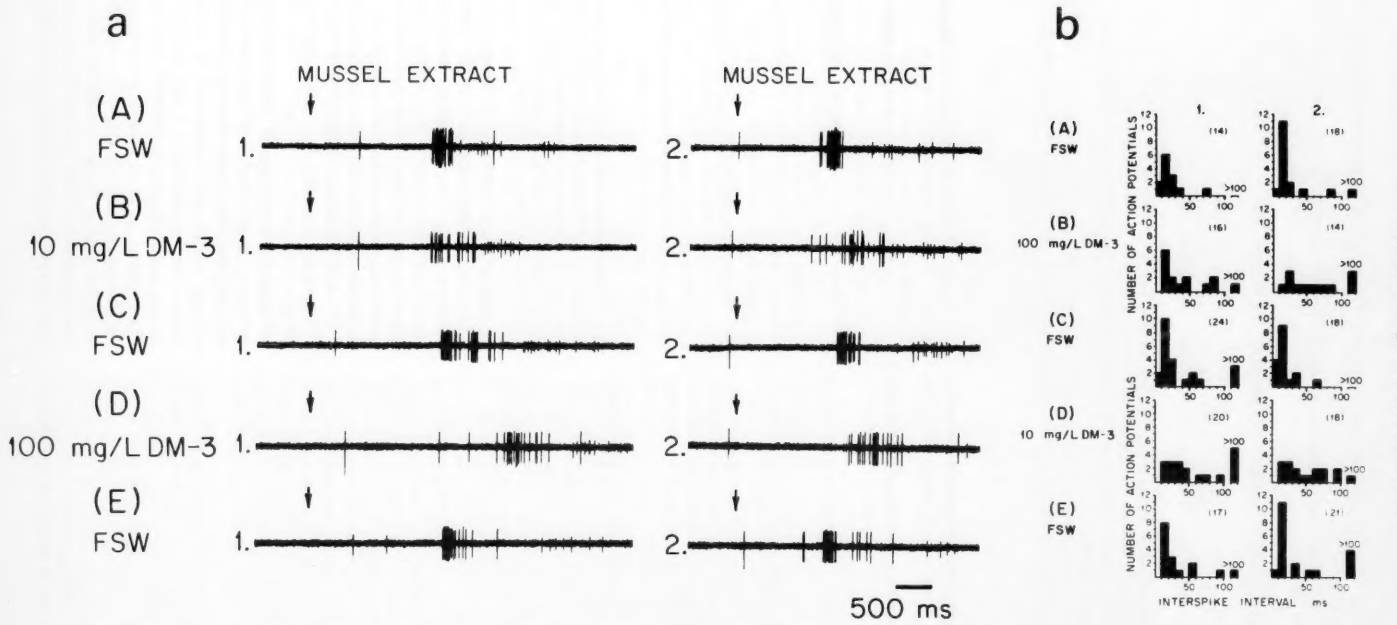

FIG. 4. Change in temporal pattern of DM-treated lobster leg chemoreceptor response to mussel extract stimulus by DM-3. (a) Recorded responses; (b) spike interval histograms of recorded responses. Mussel extract was introduced twice into each of the following background flows: (A) FSW; (B) 10 mg/L DM; (C) FSW; (D) 100 mg/L DM; (E) FSW. Both 10- and 100-mg/L DM caused increases in the mean interspike interval (spike pattern), but no reduction in number of spikes per response. The numbers in parentheses represent the total number of spikes per response.

tion, or ambiguous behavior (Percy 1976; Zimmer et al. 1979; Pearson and Olla 1979, 1980; Pearson et al. 1980, 1981).

Drilling muds can be toxic to marine crustaceans at concentrations near those used in these neurophysiological experiments. Conklin et al. (1980) examined the effects of 18 different drilling muds on two crustacean species, the grass shrimp *Palaemonetes pugio* and the mysid *Mysidopsis bahia*. When intermolt individuals of *P. pugio* were exposed to 100 mg/L drilling mud during 96-h bioassays, one mud caused 100% mortality and six caused 30−60% mortality. However, both of these species were found to be more susceptible to drilling muds during molting: 150 mg/L caused 10% mortality of *P. pugio*; for *M. bahia*, the LC_{50} for one mud was 50 mg/L, and even at 10 mg/L there was 5−25% mortality. The toxic effects of drilling muds on adult lobsters also depend on the type of drilling mud: a single dumping of one type of drilling mud, enough to form a 10-mm layer on the substrate, was lethal within 4 h of introduction (Oleszko-Szuts et al., 1979, unpublished data); other muds are less toxic (Atema personal observations).

The likelihood that marine animals in their natural habitat will be exposed to concentrations of drilling muds comparable to those used in these experiments is an important question. Dilution and dispersion rates of drilling muds released into oceanic waters are difficult to estimate since these rates depend on such variables as local atmospheric and hydrographic conditions, position of outfall with respect to the ocean bottom, and composition of drilling muds. Mariani et al. (1980), in studying the chemical and physical alterations in the benthic environment following release of drilling muds from a well off the mid-Atlantic outer continental shelf, state that most of these changes occurred within a 3-km radius of the well site. A model for a well in the Gulf of Mexico (Ray and Shinn 1975) predicts that full concentration drilling mud released at 40 bbl/h would be diluted in the open water to 1000 mg/L suspended load at 0.3 km and to 100 mg/L at 3 km downstream from the discharge; for a release rate of 250 bbl/h, a concentration of 1000 mg/L would occur in the water at 3 km downstream. However, the fact that plumes of drilling muds are often visible 4−5 km downstream from the release site (Bureau of Land Management 1979) indicates that exposure to certain components of drilling muds may be more widespread than the above studies describe. It is uncertain if it is the particulate or the soluble fraction of drilling mud which is most toxic to animals. Although many studies deal with drilling muds in solution/suspension, the amount of drilling mud covering the substrate may be an equally or more important factor for benthic animals such as adult lobsters. Under such conditions, the chemoreceptors on their legs would be chronically and directly exposed to the drilling mud.

Chemoreception is important for the ability of crustaceans to obtain such resources as mates (Atema and Engstrom 1971; Atema et al. 1979a; Gleeson 1980) and food (McLeese 1970; Reeder and Ache 1980). The sensory input from chemoreceptors on the walking legs is essential in eliciting the normal sequence of feeding behaviors (Maynard and Sallee 1970); without this input, the ability of crustaceans to locate and grasp food items is greatly impaired (Ameyaw-Akumfi 1977; Derby personal observations). Interference of the chemoreceptive systems by such pollutants as drilling muds may subsequently have consequences on the fitness of exposed animals during competition for resources.

Acknowledgments

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Predatory Feeding Behavior of *Epischura lacustris* (Copepoda, Calanoida) and Prey Defense

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WONG, C. K. 1981. Predatory feeding behavior of *Epischura lacustris* (Copepoda, Calanoida) and prey defense. Can. J. Fish. Aquat. Sci. 38: 275–279.

The predatory feeding behavior of *Epischura lacustris* on *Bosmina longirostris* and *Ceriodaphnia reticulata* was investigated. *Epischura* rarely killed *Bosmina* >0.40 mm in length, but below this threshold there was no size selection because predation rates were affected mainly by encounter rate and ingestion probability. When presented with *Bosmina* and *Ceriodaphnia*, *Epischura* fed at higher rates on *Bosmina* which were more easily captured. *Epischura* showed no true behavioral preference or switching, and its diet included all the prey it could detect, capture, and ingest successfully. Previous diet had no effect on prey selection. Predation rates on *Bosmina* were not affected by the presence of algal food.

Key words: predatory copepod, predator–prey interactions, size selection, switching, prey defense

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Le présent article décrit les résultats d'une investigation sur le comportement alimentaire d'*Epischura lacustris* vis-à-vis ses proies, *Bosmina longirostris* et *Ceriodaphnia reticulata*. *Epischura* tue rarement des *Bosmina* dépassant 0,40 mm de long. Au-dessous de ce seuil toutefois, il n'y a pas de sélection de taille, la fréquence des rencontres et la probabilité d'ingestion étant les deux principaux facteurs de régulation du taux de prédation. En présence de *Bosmina* et de *Ceriodaphnia*, *Epischura* mange davantage de *Bosmina*, plus facile à capturer. *Epischura* ne montre pas de préférence ni de changement dans son comportement, et son régime alimentaire inclut toutes les proies qu'il peut détecter, capturer et ingérer avec succès. Le régime passé n'influe pas sur la sélection des proies. La présence de nourriture algale n'agit pas sur le taux de prédation chez *Bosmina*.

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RECENT studies have shown that size-selective feeding by invertebrate planktivores may determine the structure and size composition of freshwater zooplankton communities (Brooks and Dodson 1965; Dodson 1974; Kerfoot 1975). Many authors have observed that zooplankton predators prefer small zooplankters to large ones (Anderson 1970; Brandl and Fernando 1975, 1978; Confer 1971; Confer and Blades 1975; Kerfoot 1977; McQueen 1969), but few of the data were obtained by giving the predators a choice, and the mechanisms of the selective process were not thoroughly analyzed. Although several recent studies (Gerritsen 1978; Kerfoot 1978; Li and Li 1979) have stressed the importance of swimming behavior and morphology to prey susceptibility, it is generally assumed that size will determine the selection pattern for prey similar in these characteristics.

Switching may be defined as the ability of a predator to take a disproportionately large number of the most abundant prey species (Murdoch 1969). Natural selection favors predators that can, as a result of learning or physiological changes, switch quickly to an alternate food when one food type becomes scarce. Since switching exerts a stabilizing effect on the prey populations (Murdoch 1969), it is important to determine whether swithcing is a normal feature of copepod predation. Information on switching and the effect of previous diet on the feeding behavior of copepods is available for filter-feeding marine species (Barnett 1974; Conover 1966; Donaghay 1978; Lonsdale et al. 1979; Mullin 1979), but lacking for freshwater species.

In this paper I report on size selection by *Epischura lacustris*, a predaceous calanoid copepod, on the cladoceran, *Bosmina longirostris*. My first objective is to determine the relationship between size and vulnerability in a prey which does not undergo major changes in swimming behavior and morphology during ontogeny. Secondly, I will investigate switching by *Epischura* on two cladoceran species (*Bosmina longirostris* and *Ceriodaphnia reticulata*) and the role of previous diet on prey selection. Since *Epischura* adults are omnivorous (Friedman 1977; Main 1962), the effects of the pres-

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ence of algae on the predation rate of *Epischura* is also studied.

Materials and Methods

Epischura lacustris was collected once a week from Pinks Lake, Quebec. *Bosmina longirostris* was obtained from laboratory cultures of specimens collected from Pinks Lake 1 yr before the experiments began. *Ceriodaphnia reticulata* was collected from Heney Lake, Quebec, a few weeks before they were used. Preliminary tests indicated that *Epischura* could not tolerate starvation; therefore, individuals were kept in a 40-L aquarium at 20°C with other zooplankton from Pinks Lake.

All selection experiments were conducted in 600-mL plastic tissue culture bottles containing filtered lake water from Pinks Lake. Bottles were positioned horizontally and placed in darkness at 20°C. Adult *Epischura* were transferred from the aquarium to small beakers 15–30 min before the experiments began. Prey of different sizes and developmental stages were selected with a stereomicroscope. A single *Epischura* was added to the bottle after the prey were introduced. All experiments lasted for 4–5 h. At the end of each experiment the water was filtered through 110-μm plankton netting and the prey were counted under a stereomicroscope. Recovery of prey was always 100%. Since *Epischura* ingests *Bosmina* without seriously damaging the carapace, the bodies of attacked *Bosmina* are easily distinguishable from molted exuviae. At the end of the experiment the body length of each live and killed *Bosmina* was measured. For *Ceriodaphnia*, each missing individual was presumed to have been consumed by *Epischura*.

To determine whether the presence of phytoplankton affects the predation rate of *Epischura*, *Bosmina* were offered to the predator with various concentrations of laboratory cultured *Ankistrodesmus* spp. (Woods Hole HBL medium, Wayne Nichols 1973). Algae were centrifuged and resuspended in filtered lake water before being used. Algal concentrations were determined by counting at least 500 cells in a hemacytometer. The experiments were carried out in bottles wrapped in black plastic and postioned on a plankton wheel (1.5 rpm). To study the training effects of previous diet, *Epischura* were kept in *Ankistrodesmus* suspension for 24 h before *Bosmina* were introduced to the bottle. Since algal concentrations may change during training, it should be noted that concentrations plotted below are those at the beginning of the training period.

Direct observations of *Epischura* attacking prey were made with a stereomicroscope. An adult *Epischura* starved for about 20 h was transferred into 20 mL of filtered lake water in a 50-mm-diameter Petri dish containing 20–40 *Bosmina* of various sizes. *Epischura* usually recovered from the handling procedure within 2–3 min and began to attack prey. An attack was scored if the predator had grasped the prey with its mouthparts. Predators that did not attack prey in 3 min were replaced. All attacked *Bosmina*, whether alive or killed, were fixed in 10% formalin and measured.

Zooplankton swimming speeds were determined by observing animals in a 50-mm-diameter Petri dish or in a 90 × 40 × 15 mm tank fitted with a grid of 1-mm squares. The time required to traverse 10 squares was recorded with a stop watch.

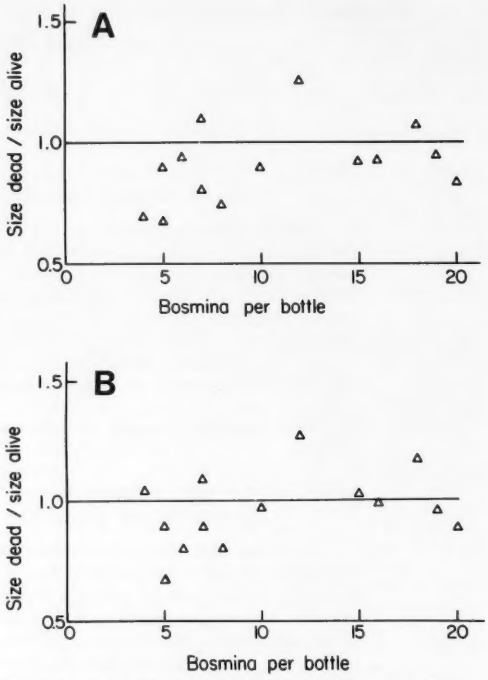

FIG. 1(A). Size selection by *Epischura* on *Bosmina* at different prey densities; (B) based on the same data except all *Bosmina* >0.40 mm are excluded from the calculations. Each point represents a separate experiment.

Results and Discussion

Bosmina

Bosmina picked randomly from the cultures to obtain a mixture of sizes were offered to *Epischura* at densities of 4 to 20 individuals per bottle. The mean sizes of the live *Bosmina* were larger than those of the killed *Bosmina* (Fig. 1A) (Wilcoxon matched-pairs signed-ranks test, $P = 0.03$). However, *Bosmina* >0.40 mm were rarely killed, and the smallest *Bosmina* were killed in only 6 of the 14 experiments. When the results were analyzed again, excluding all *Bosmina* >0.40 mm (Fig. 1B), the difference between the mean sizes of the live and the killed *Bosmina* was not significant (Wilcoxon match-pairs signed-ranks test, $P = 0.18$). When five *Bosmina* <0.25 mm were offered to *Epischura* together with five *Bosmina* >0.40 mm, the predation rate on the small *Bosmina* was higher than that on the large ones (Table 1, expt. 1).

Epischura rarely killed *Bosmina* >0.40 mm and those few that were killed were never completely consumed. There seems to exist an upper limit of prey size which is determined

by the mouthpart morphology of the predator. Kerfoot (1978) suggested that because *Epischura* uses the swimming legs to form part of the floor of the feeding chamber, it cannot securely grasp a *Bosmina* which is longer than the distance between its first swimming legs and its first antennae. In *E. lacustris* the length of the feeding chamber averages 0.43 mm ($n = 10$) for males and 0.47 mm ($n = 10$) for females. Therefore, a *Bosmina* larger than 0.40 mm and with a mucro (average length 0.02−0.04 mm) would be difficult for *Epischura* to manipulate.

To study size selection when *Epischura* is offered prey of manageable sizes, *Bosmina* <0.25 mm and those of 0.28−0.35 mm were offered in a 1:1 ratio. The pattern of size selection was studied at prey densities of 4, 10, and 20 *Bosmina* per bottle. *Epischura* did not select *Bosmina* on the basis of size at any of the prey densities studied (Table 1, expt. 2). To determine whether *Epischura* shows selection when the proportion of one size-class in the prey population increases, *Bosmina* of the same two size-classes were offered in ratios of 1:3 and 3:1 with prey density kept at 20 prey per bottle. *Epischura* preyed upon the two size-classes according to the ratios at which they were offered (Table 1, expt. 3).

Bosmina of various sizes avoid an attacking *Epischura* by sinking passively (Kerfoot 1977, 1978). Direct observations of *Epischura* attacking *Bosmina* reveal that the predator will attack prey of any size, but the smaller the *Bosmina*, the greater the probability of ingestion after being grasped by the copepod (Table 2). Since *Bosmina* <0.25 mm and *Bosmina* of 0.28−0.35 mm were preyed upon at the same rate, the larger *Bosmina* must have encountered the predator at a higher rate than their smaller counterparts. Both large and small *Bosmina* swim at about 1.5 mm/s; therefore, the swimming speed does not explain the different encounter rates. *Epischura* detects prey through mechanical disturbances in the water (Strickler and Twombly 1975). Assuming the amount of disturbance caused by a swimming *Bosmina* increases with the size of the animal, then the larger the *Bosmina*, the greater the distance from which it is detected by *Epischura*. Hence, large *Bosmina* have low ingestion probability, but this advantage is counteracted by a high encounter rate.

TABLE 1. Size-selective predation by *Epischura* on *Bosmina*.

Expt.	Prey/ bottle		Replicates	Total prey killed	Ratio Expected		: Observed		
1	L[b]	S[c]			L	S	L	S	
	5	5	4	12	6	6	1	11	s[a]
2	I[d]	S			I	S	I	S	
	2	2	9	23	11.5	11.5	12	11	ns
	5	5	9	21	10.5	10.5	13	8	ns
	10	10	8	39	19.5	19.5	24	15	ns
3	I	S			I	S	I	S	
	5	15	6	57	14.25	42.75	15	42	ns
	15	5	5	29	21.75	7.25	22	7	ns

[a]ns = not significant; s = significant by Chi-square goodness-of-fit test at $P = 0.05$.
[b]*Bosmina* >0.40 mm.
[c]*Bosmina* <0.25 mm.
[d]*Bosmina* 0.28−0.35 mm.

TABLE 2. Ingestion probabilities for *Bosmina* of various sizes after being grasped by *Epischura* (L = *Bosmina* from laboratory culture, F = *Bosmina* collected from Pinks Lake on June 25, 1979).

Bosmina size (mm)		No. of attacks observed	No. (probability) ingested
0.25	L	21	14 (0.67)
	F	18	6 (0.33)
0.26−0.30	L	8	1 (0.13)
	F	23	2 (0.09)
0.31−0.35	L	7	0 (0.00)
	F	7	0 (0.00)

Bosmina AND *Ceriodaphnia*

To examine prey selection and switching, *Epischura* was offered two prey species of similar size (0.28−0.35 mm) but differing morphology. Adult *Bosmina* and first instar *Ceriodaphnia* were presented in different ratios with the total prey density kept at 20 prey per bottle. The preference for a given prey is assessed by using the logarithm of Jacob's (1974) modified version of the forage ratio

$$Q = r(1 - p)/p(1 - r)$$

where r is the fraction in the diet and p is the fraction in the environment. At each prey ratio, r for a given prey is determined by using the total number of each prey killed over eight replicates. A value of $\log Q > 0$ indicates positive selection while a value <0 indicates negative selection. *Epischura* selected *Bosmina* over *Ceriodaphnia* at all prey ratios (Fig. 2). To study the effect of previous diet the experiments were repeated using *Epischura* that had been exposed to either *Bosmina* or *Ceriodaphnia* for a 24-h training period at a prey density of 10 prey per bottle. Only predators that had killed at least one prey during the 24-h period were used. The results indicate that previous diet did not alter the predator's preference for *Bosmina*. A regression analysis was performed on each of the three sets of data (untrained, *Bosmina*-trained, and *Ceriodaphnia*-trained). An analysis of covariance shows that the three regression lines are statistically identical ($F = 0.07$; df = 2,11; $P > 0.05$). The results appear spurious because of the high variability among replicates, however, because the slopes of all three regression lines do not differ from zero (t-test), *Epischura* did not show any switching.

Ceriodaphnia hangs motionless in the water for a long period between swimming bouts. This swimming behavior gives it a mean speed of 1.5−2.5 mm/s, which is slighly higher than that for the continuously swimming *Bosmina* (1.5 mm/s). These differences in swimming behavior mean that *Ceriodaphnia* has a higher encounter rate with *Epischura* than *Bosmina* (Zaret 1979). Since *Bosmina* is preyed upon at a higher rate than *Ceriodaphnia*, encounter probability is not the main factor that determines prey selection here. *Ceriodaphnia* perceives an approaching predator from as far as 3 mm, whereas *Bosmina* senses a predator only at close range (<1 mm). *Epischura* searches for its prey by swimming in somersault-like patterns in the disturbed area (Kerfoot

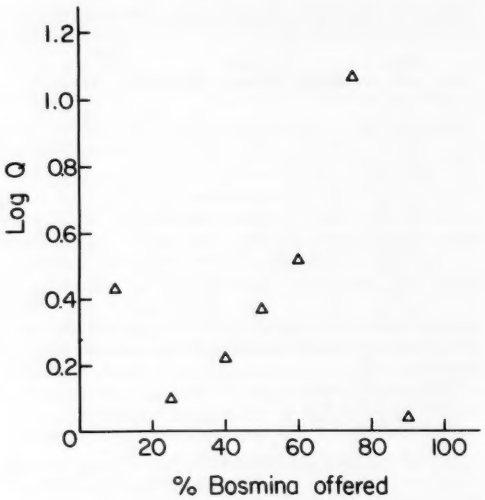

FIG. 2. Logarithm of the forage ratio (Q) for *Bosmina* by *Epischura* as a function of the percentage of *Bosmina* offered. *Ceriodaphnia* was the alternative prey (Regression: $y = 0.278 - 0.003x$, $t = -0.46$, df = 5, $P > 0.25$).

1978). This searching behavior is successful with *Bosmina* which escapes by sinking vertically, but becomes ineffective with *Ceriodaphnia* which flees by swimming away quickly along a straight path in any direction. When captured, *Bosmina* is partially protected by its carapace and mucro. On the other hand, *Ceriodaphnia*, whose carapace is easily torn apart by *Epischura*, may rely on its spherical shape, which makes it difficult to grasp, to frustrate the predator. The ability of *Ceriodaphnia* to escape after being captured was not determined, but there is little doubt that *Epischura* eats more *Bosmina* because it is easier to capture.

OMNIVOROUS FEEDING

The predation rate of *Epischura* on *Bosmina* (10 per bottle, 0.28−0.35 mm) did not change with increasing concentrations of *Ankistrodesmus* (Fig. 3). However, when *Epischura* was exposed to *Ankistrodesmus* for 24 h prior to the introduction of prey, the predation rates for those trained to high algal concentrations were lower than the rates for those trained to low algal concentrations (Fig. 4).

The presence of algae does not alter the predation rate of *Epischura* on *Bosmina*. The feeding habits of early stages of *Epischura* have not been studied, but nauplii are undoubtedly herbivorous, while copepodites and adults are omnivorous (Friedman 1977; Main 1962). I observed that the guts of *Epischura* adults were green after a brief exposure to algae. However, since about 50% of the animals did not survive 24 h in algae, it appears that *Epischura* cannot utilize algal food efficiently. Main (1962) found that with the exception of *Pediastrum*, algal foods are not sufficient to sustain growth and reproduction in *Epischura*. Corner et al. (1976) found that

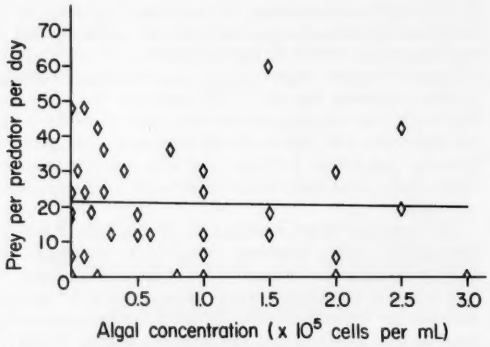

FIG. 3. Predation rate by *Epischura* on *Bosmina* in the presence of different concentrations of *Ankistrodesmus*. Each point represents a separate experiment (Regression: $y = 21.47 - 0.418x$, $t = -0.14$, df = 43, $P \gg 0.05$).

the marine copepod *Calanus*, when feeding as a carnivore, assimilates its food at a higher efficiency than when feeding on algal diets. If algal foods are incomplete or lower in nutritional value than animal foods, then the presence of algae would not affect carnivorous feeding in *Epischura*. Londsdale et al. (1979) suggested that small algae do not reduce the predation rate of adult *Acartia tonsa* on copepod nauplii because the predator prefers the large nauplii to the small algal cells and the feeding on small algae does not interfere with the handling of prey. Since Main (1962) found that *Epischura* adults feed at a higher rate on large algae than on small ones, *Ankistrodesmus* (3−30 μm) may not be suitable for *Epischura*. *Epischura* that had been trained to high concentrations of algae reduces its predation rate on *Bosmina*. This could be the result of satiation; however, the high mortality rate of *Epischura* (>75% when algal concentration $>2 \times 10^5$ cells/mL) suggests that high concentration of *Ankistrodesmus* may have had some adverse effects on the predator.

Conclusion

The results presented in this paper show that *Epischura* cannot handle *Bosmina* >0.40 mm efficiently, but when offered *Bosmina* <0.40 mm, there is no size selection. This suggests that for a prey species whose shape and swimming behavior do not change much with growth, susceptibility to predation still may not decrease with increasing size.

Epischura is not capable of any active choice. Selectivity reflects (1) the ability of *Epischura* to detect, capture, and ingest a given prey species, and (2) species differences in the effectiveness of prey defense. *Epischura* has low tolerance to starvation (mortality was always >50% after 24 h without food) and has a high predation rate (e.g. 40 prey per predator per day). For a predator, the net value of a prey is its gross caloric value minus the cost in terms of the energy required to search for and handle it. Since the handling time for prey is low (2−3 min for *Bosmina*), *Epischura* spends most of its time searching for prey. Searching time decreases when the number of prey types in the diet increases (MacArthur and Pianka 1966). If active selective predation is too costly in

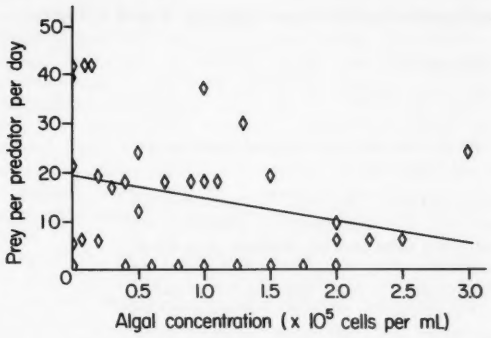

FIG. 4. Predation rate by *Epischura* on *Bosmina* in the presence of different concentrations of *Ankistrodesmus*. *Epischura* trained to *Ankistrodesmus* at concentrations equal to the experimental concentrations. Each point represents a separate experiment (Regression: $y = 19.21 - 4.59x$, $t = -2.07$, df = 33, $P < 0.05$).

terms of time, then *Epischura* may be more efficient by being a jack-of-all-trades (Kerfoot 1975), eating whatever it can detect, capture, and ingest.

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Equilibrium Model of Fate of Microcontaminants in Diverse Aquatic Food Chains

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THOMANN, R. V. 1981. Equilibrium model of fate of microcontaminants in diverse aquatic food chains. Can. J. Fish. Aquat. Sci. 38: 280–296.

Bioconcentration and bioaccumulation factors of PCB, ^{239}Pu, and ^{137}Cs are compiled from the literature as a function of organism size. The distribution of field-observed bioaccumulation factors varies markedly between each substance but similarly to order of magnitude within each substance across diverse food chains. It can be inferred from the literature that PCB levels in top predators are due primarily to food chain transfer. A steady state compartment food chain model is derived for estimation of the relative effect of uptake directly from water versus food chain transfer. The model food chain transfer number f, given by $\alpha C/K + G$ for α = chemical absorption efficiency, C = specific consumption, K = excretion rate, and G = net organism growth rate indicates the degree of food chain accumulation. For $f > 1$, food chain transfer is significant; for $f < 1$, uptake from water is more significant. Application of the model suggests that (a) PCB body burden in top predators is due almost entirely to consumption of contaminated prey, (b) for ^{239}Pu all of the body burden is due to uptake from the water only, and (c) observed ^{137}Cs concentration factors are due principally to food chain transfer with a high dependence on the salinity-dependent phytoplankton adsorption.

Key words: food chain model, bioconcentration, bioaccumulation, PCB, ^{239}Pu, ^{137}Cs, water uptake, food chain transfer

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L'auteur a compilé les facteurs de bioconcentration et de bioaccumulation des BPC, de ^{239}Pu et de ^{137}Cs en fonction de la taille des organismes, à partir des données publiées. La répartition des facteurs de bioaccumulation observés sur le terrain varie de façon marquée d'une substance à l'autre, mais aussi en ordre de grandeur, pour une même substance, à travers une chaîne alimentaire diversifiée. On peut déduire des publications que les niveaux de BPC chez les prédateurs supérieurs sont dus principalement au transfert dans la chaîne alimentaire. On a établi un modèle de chaîne alimentaire compartimenté et en état d'équilibre dans le but d'estimer l'effet relatif d'une absorption directe de l'eau versus le transfert dans la chaîne alimentaire. L'indice de transfert dans la chaîne alimentaire f, déduit de $\alpha C/K + G$ où α = l'efficacité d'absorption chimique, C = la consommation spécifique, K = le taux d'excrétion et G = le taux de croissance net de l'organisme, indique le degré d'accumulation dans la chaîne alimentaire. Pour $f > 1$, le transfert dans la chaîne alimentaire est significatif; pour $f < 1$, l'absorption à partir de l'eau est plus importante. L'application du modèle laisse penser que: (a) la charge corporelle de BPC chez les prédateurs supérieurs provient presque exclusivement de la consommation de proies contaminées, (b) quant à ^{239}Pu, toute la charge corporelle est due seulement à l'absorption à partir de l'eau et (c) les facteurs de concentration de ^{137}Cs observés résultent principalement du transfert dans la chaîne alimentaire, avec assujetisse.nent à l'absorption de phytoplancton dépendante de la densité.

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INFORMATION on microcontaminants in aquatic food chains has accumulated over a period of years for a wide variety of water bodies throughout the world. Such data are currently being collected at an ever increasing rate as a result of heightened concern over environmental deterioration. Examples of such concern include PCB contamination of the Hudson River and Great Lakes fisheries (Hetling et al., 1978; Delfino 1978), studies of insecticides, PCB, and metals in African lakes (Greichus et al., 1978) and long-term monitoring of chlorinated hydrocarbons in Lake Paijanne, the second largest

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lake in Finland (Hattula 1978). Concurrently, there is an urgent need to describe cause and effect relationships between discharges of toxic substances and resulting levels in the aquatic food chain. An attempt is made here to collate and evaluate data on potentially hazardous substances using a simplified transfer model. The objective of this work therefore is to use a simple model of chemical distribution in aquatic food chains to estimate expected levels in aquatic organisms under different cases of chemical bioaccumulation. Ultimately, a basis may be provided for the allocation of chemical discharges to meet specified target levels in the food chain.

In addition, there is considerable confusion over the extent to which food chain transfer of chemicals influence the potential for bioaccumulation. For example, Scura and Theilacher (1977) in evaluating laboratory data on transfer of chlorinated hydrocarbons (CHC) with an algal flagellate, rotifer, and anchovy larva food chain concluded that "CHC accumulation is not a food-chain phenomenon but rather the result of direct partitioning of the compounds between the seawater and the test organisms." Macek et al. (1979) in a summary of experimental data concluded that with the exception of DDT, "the process of biomagnification, as classically defined, probably does not occur within communities of aquatic organisms." These evaluations of the significance of food chain accumulation, it should be noted, resulted from laboratory microcosm data of simplified food chains that did not include natural top predators. On the other hand, Weininger (1978) using a model of PCB accumulation in the lake trout of Lake Michigan and comparing the model to observed field data concluded that "Direct uptake of PCBs can only account for 2−3% of total observed PCB accumulation by adult lake trout, although this route is more important for juveniles." These contradictory reports are due in part to the lack of a suitable modeling framework to provide a basis for estimating the relative effect of food chain transfer versus direct uptake from the water.

My intentions are to:

1) collate published data gathered from different ecosystems to search for unifying trends in fate of microcontaminants;
2) present evidence strongly indicative of food chain transfer for a toxicant such as PCB and conversely to present evidence for lack of importance of food chain transfer for certain chemicals;
3) develop a simple food chain model of contaminant transfer;
4) apply the model to some data on chemicals with varying behavior in aquatic ecosystem.

Size-Dependent Food Chain Bioaccumulation

The existing information on microcontaminants in various sectors of the food chain can be organized in a convenient way by relating the toxicant concentration to organism size (or through appropriate allometric relationships to organism wet weight). The use of some measure of the length of an organism serves as a metric for displaying food chain relationships although it is recognized that the size of an organism is not necessarily a good general measure of trophic position (Thomann 1978, 1979). Organism size may be defined in terms of the spherical equivalent of a known mass as in Sheldon et al. (1972) or in this paper simply as a representative size to the nearest order of magnitude. The contaminant concentration can be normalized between ecosystems with the water concentration, by defining

$$(1) \qquad N'(L) = \frac{v'(L)}{c}$$

where v' is the concentration of the toxicant in the organism (μg/g(d))(g(d) = grams dry weight), c is the concentration of the toxicant in the water (μg/g), L is the organism size (μm), and N' is the concentration factor. The difficulty with this normalization is the uncertainty of the water concentration of the microcontaminant. At the low levels of certain contaminants such as the chlorinated hydrocarbons, the analytical difficulty is substantial and analyses from laboratory to laboratory are often inconsistent. For other contaminants, however, such as the radionuclides discussed below, analytical techniques have been developed over several decades and the water concentrations are known with considerable more assurance than contemporary toxicants. The concentration factor on a wet weight basis is given by

$$(2) \qquad N(L) = \frac{v(L)}{c}$$

where v is now μg/g(w), (g(w) = grams wet weight).

Note that

$$(3a) \qquad v'(L) = \beta(L)v(L)$$

and

$$(3b) \qquad N'(L) = \beta(L)N(L)$$

where β is the ratio of wet weight to dry weight. For this work, this ratio was set at 10 (90% water) for the phytoplankton, 5 for the zooplankton, and 4 for the upper levels of the food chain. In Eq. (1) and (2), the water concentration is usually an operationally defined "dissolved" portion (e.g. <0.45 μm or after centrifugation).

A distinction is also necessary between the experimental bioconcentration of a chemical directly from the water, designated herein as N'_w and the bioaccumulation of the chemical under natural field conditions designated N'_F. The latter factor includes the accumulation of the contaminant directly from the water as well as from feeding habits on prey with varying levels of contamination. In general then

$$(4) \qquad \frac{N'_F(L)}{N'_w(L)} \geq 1.$$

If $N'_F = N'_w$, then the effect of food chain transfer is zero, and if $N'_F \gg N'_w$, then the uptake from food consumption must be important. It is useful to compare N'_F to N'_w for different-sized organisms and for several distinct aquatic ecosystems to determine from the data alone whether food chain transfer is of significance. PCB, ^{239}Pu, and ^{137}Cs were chosen to illustrate the varying importance of food chain uptake and because of the existence of a considerable worldwide data base.

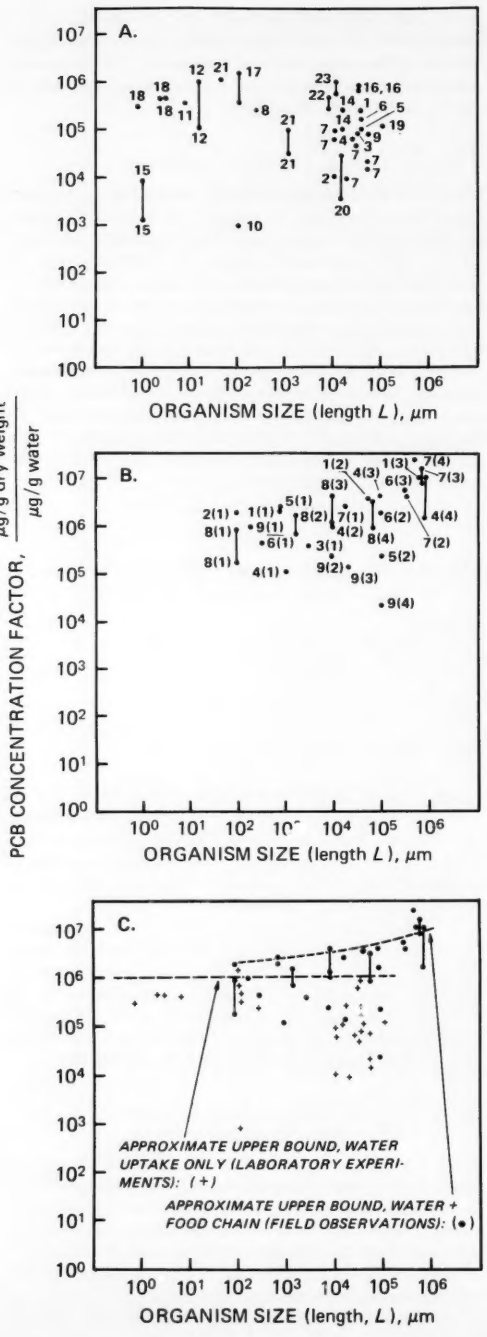
A.
B.
C.
PCB CONCENTRATION FACTOR, μg/g dry weight / μg/g water
ORGANISM SIZE (length L), μm
APPROXIMATE UPPER BOUND, WATER UPTAKE ONLY (LABORATORY EXPERIMENTS): (+)
APPROXIMATE UPPER BOUND, WATER + FOOD CHAIN (FIELD OBSERVATIONS): (•)

FIG. 1(a). PCB bioconcentration factor, (dry weight basis) uptake from water only, see Table 1 for references; (b) observed PCB bioaccumulation factors (dry weight basis) from field measurements; uptake from water and food, see Table 2 for references; (c) comparison of PCB concentration factors from water only to PCB concentration factors under field conditions.

SIGNIFICANCE OF FOOD CHAIN ACCUMULATION OF PCB

The observed behavior of $N'_w(L)$, the bioconcentration due only to uptake of PCB directly from the water, and $N'_F(L)$, the bioconcentration under natural field conditions is plotted against organism size in Fig. 1. (See Tables 1 and 2 for supporting references.)

Figure 1A shows $N'_w(L)$ as calculated from the literature. The scatter is large and no discernible trend is evident. All of the data shown in Fig. 1A were obtained from laboratory aquaria experiments, sometimes not under the best of controlled conditions. Data were used only where the experiment was judged to have reached equilibrium and where it appeared that the reported water concentration was consistent with the above definition of the dissolved fraction. For some experiments, the dissolved fraction was estimated from the total water concentration. In those instances, additional suspended solids may have been generated during the test (through fecal or particle formation, for example) which may have lowered the dissolved component such that N'_w would be overestimated. It should be noted too, that the largest organism used in these experiments is $\sim 10^5$ μm (0.1 m). There is no information on PCB uptake from water for top carnivores such as lake trout or striped bass. With a few exceptions, the experiments used the higher chlorinated isomers or mixtures of PCB, e.g. Aroclor 1254. The approximate upper bound for the bioconcentration factor due to water alone from Fig. 1A is about 10^6 μg/g(d) $\div$ μg/g.

Figure 1B shows on comparable scales, the PCB bioaccumulation factors for different-sized organisms from field data for nine water bodies. The greatest uncertainty in the above values is due to the estimate of the dissolved fraction, c. For some areas a reasonable range of values is at hand (e.g. Hudson Estuary and Western Lake Superior). For other regions, e.g. Milwaukee Harbor, an estimate was made based on equilibrium partitioning to a known or assumed level of suspended solids. Figure 1B does appear to indicate an upward trend toward higher $N'_F(L)$ as one proceeds up the food chain. A notable exception is the data for the Mediterranean Sea off the French coast (no. 9 in Fig. 1B) reported by Fowler and Elder (1978). The PCB in that food chain clearly decreases from the microplankton (no. 9 (1)) to the myctophid fish (no. 9 (4)). The upper bound of the data in Fig. 1B, however, indicates a general increase from about 10^6 for the microplankton (100 μm) to greater than 10^7 for the upper levels of the food chain.

Figure 1C combines 1A and B to show more clearly the increasing divergence between N'_F and N'_w indicating an accumulation of PCB with trophic level. At 10^6 μm, for example, the upper bound in the field data is about 10^7 or one order of magnitude greater than the amount of PCB that would be accumulated as a result of uptake from the water alone.

TABLE 1. Explanation of symbols for Fig. 1(a). PCB concentration factors, water only.

No.	Organism or particle type	PCB analyzed	Reference
1.	Spot (*Leiostomus xanthurus*)	Arclor 1254	Moriarty (1975)
2.	Mayfly nymph (*Ephemera danica*)	Clophen A 50	Sodergren and Svensson (1973)
3.	Oyster (*Crassostrea virginica*)	Isomer mixture	Vreeland (1974)
4.	Pinfish (*Lagodon rhomboides*)	Arcoclor 1016	Hansen et al. (1974)
5.	Spot (*Leiostomus xanthurus*)	Aroclor 1254	Duke and Dumus (1974)
6.	Minnow (*Cyprinodon variegatus*)	Aroclor 1254	Hansen et al. (1973)
7.	Aquatic invertebrates; midge, scud, mosquito larvae, stonefly dobsonfly, crayfish	Aroclor 1254	Sanders and Chandler (1972)
8.	Cladoceran (*Daphnia magna*)	Aroclor 1254	Sanders and Chandler (1972)
9.	Pink shrimp (*Penaeus duorarum*)	Aroclor 1254	Nimmo et al. (1971)
10.	Protozoa (*Tetrahymena pyriformis*)	Aroclor 1254	Cooley et al. (1972)
11.	Algae (*Chlorella*)	Clophen A 50	Sodergren (1977)
12.	Marine phytoplankton — natural assemblage	Isomer mixture	Biggs et al. (In prep.)
13.	Illite clay, Woodburn soil	Aroclor 1254	Haque et al. (1974)
14.	Amphipod (*Gammarus*)	Aroclor 1242	Nebeker and Puglisi (1974)
15.	Bacteria, seston and sediment	Aroclor 1016 and 1242	Paris et al. (1978)
16.	Fathead minnow (*Pimephales promelas*)	Aroclor 1242 and 1248	Nebeker et al. (1974)
17.	Centric diatoms	Pentachlorobiphenyl	Harding and Phillips (1978)
18.	Nuchar-Attaclay, sewage microbes, natural particulates	Pentachlorobiphenyl	Harding and Phillips (1978)
19.	Rainbow Trout (*Salmo gairdneri*)	2,2′,4,4′-Tetra-chlorobiphenyl	Branson et al. (1975)
20.	Killifish (*Fundulus heteroclitus*)	Tetrachlorobiphenyl	Sugiura et al. (1978)
21.	Copepods (*A. Tonsa*) (*A. Clausi*)	^{14}C-labeled Aroclor 1254	Wyman and O'Connors (1979)
22.	*Skelotonenema*	^{14}C-labeled Aroclor 1254	Wyman and O'Connors (1979)
23.	Fathead minnow (*Pimephales promelas*)	Aroclor 1248 Aroclor 1260	DeFoe et al. (1978)

Additional data are available on PCB levels in top carnivores through extensive field sampling of PCB content in the striped bass of the Hudson Estuary conducted by the New York State Department of Environmental Conservation (NYS DEC) (see Hydroscience 1979). A total of 371 striped bass (largely in the 4−5 year-class) have been analyzed from spring collections during the bass migration period. Quality control was excellent. Three Aroclors were analyzed (1221, 1016, 1254) together with measurements of fresh weight, length, and % lipid for each fish.

Figure 2 shows the cumulative frequency distribution of the log PCB of the Hudson striped bass. The break in the distribution at the 10% level indicates that the density function is bimodal. This may reflect two populations of striped bass; resident and migratory. The median level for the entire population is 10 μg/g(w), but note that the 5% level is 70 μg/g(w), and 2½% of the total population sampled had concentrations of greater than 100 μg/g(w). These latter levels are therefore 7 to 10 times higher than the median level. Also shown in Fig. 2 is the concentration factor for a range of dissolved estuary and near-shore water concentration of 0.01−0.05 μg/L (Hydroscience 1979). A dry to wet conversion factor of four was used. Fifty percent of the fish therefore had concentration factors of $10^{5.9}-10^{6.6}$ or greater. Five percent of the fish had concentration factors of at least $10^{6.75}-10^{7.45}$. These levels are 0.5−1.5 orders of magnitude higher than the levels that have been reported for uptake directly from the water. The difference is ascribed to feeding habits, exposure to higher water concentration than assumed, or a combination of the two. These results indicate the high probability that when a sufficient number of samples is taken, a toxic substance such as PCB can be accumulated to levels significantly greater than would be estimated from laboratory or microcosm experiments.

THE BIOACCUMULATION OF PLUTONIUM-239

It is desirable to test and apply the general applicability of any model to as wide a range of conditions as possible. Accordingly, a search was made for microcontaminants that

TABLE 2. Explanation of symbols for Fig. 1(b). PCB bioaccumulation factors, field observed.

Number	Location and organism,	Reference
	Lake Ontario	
1(1)	Net plankton (>64 μm)	Haile et al. (1975)
1(2)	Alewife	Risebrough (1976)
1(3)	Coho	Veith (1976a)
	Milwaukee River	
2(1)	Net plankton	Veith (1970)
	Puget Sound	
3(1)	Zooplankton	Pavlou and Hom (1978)
	Hudson River Estuary	
4(1)	Macroinvertebrates	Hetling et al. (1978)
4(2)	Small (<300 μm) fish	Hydroscience (1978)
4(3)	Largemouth bass (*Micropterus salmoides*) Adult striped bass (*Morone saxatilis*)	Hydroscience (1979)
	N.W. Med. (Ligurian Sea)	
5(1)	Microplankton	Elder and Fowler (1977)
5(2)	Euphausiid	
	Western Lake Superior	
6(1)	Zooplankton (*Mysis relicta*)	Veith et al. (1977)
6(2)	Sculpin	Swain (1977)
6(3)	Pelagic fish	
	Lake Michigan	
7(1)	*Pontoporeia affinis*	Peterson and Ellarson (1978)
7(2)	Bloater chub (*Hybopsis plumbea*)	Swain (1978)
7(3)	Coho (*Oncorhynchus kisutch*) and lake trout (*Salmelinus namaycush*)	Weininger (1978)
	Oldsquaw duck (*Clangula hyemulis*)	Peterson and Ellarson (1978)
	South Swedish Lake	
8(1)	Seston	Sodergren (1973)
8(2)	Zooplankton (>200 μm)	
8(3)	Invertebrates, leeches, detritus feeders	
8(4)	Fish	
	Med. Coast France	
9(1)	Microzoopl. (>76 μm)	Fowler and Elder (1978)
9(2)	Euphausiid	
9(3)	Shrimp	
9(4)	Myctophid fish	

would display markedly different behavior than PCB. Information on ^{239}Pu, a radionuclide generated from weapons testing, provides such an example. A considerable effort has been expended worldwide in measuring the bioaccumulation of ^{239}Pu between the water column, sediments, and biota of major water bodies. The laboratory data base on bioconcentration of ^{239}Pu from the water, however, is not as extensive, and a review is given under Model Calibration. The general trend of the ^{239}Pu bioaccumulation factor is downward as one proceeds up the food chain (Fig. 3, Table 3). A decrease of approximately four orders of magnitude is observed from the lower levels of the food chain ($N = 10^4$) to the top predator of 10^6 μm ($N = 10^0$). At any level in the food chain, the variation is about one to two orders of magnitude, but in all ecosystems the general downward trend is similar.

THE BIOACCUMULATION OF CESIUM-137

Field values for Cesium-137 content in organisms provide a final example (Fig. 4, Table 4). The influence of salinity on the cesium uptake is pronounced. The bioaccumulation factors are reduced in saline water due to the higher concentrations of potassium, which is similar in chemical structure to cesium. Vanderploeg et al. (1975) have compiled information on water uptake of cesium and have discussed and quantified the effect of different potassium levels on cesium

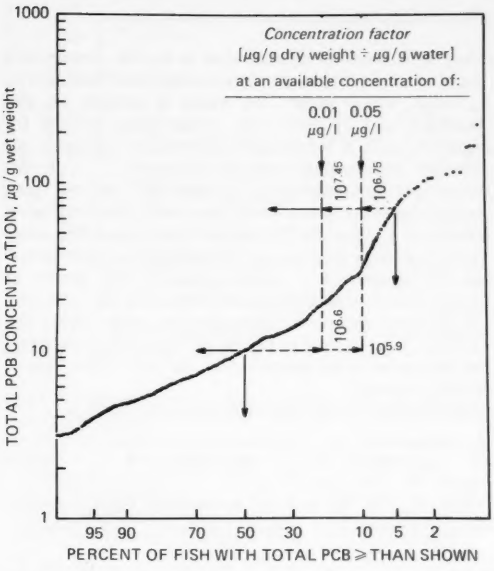

FIG. 2. Frequency distribution of log PCB of Hudson Estuary striped bass, spring 1978 (Data of New York State, Dept. of Env. Cons.).

uptake. The bioconcentration of ^{137}Cs is reviewed under Model Calibration.

The general shape of the cesium food chain distribution represents an intermediate between PCBs and ^{239}Pu. Freshwater concentration factors vary from about 10^3 at the phytoplankton level to about 10^4 at the top predator level indicating some potential for bioaccumulation. It is rather interesting that discrete ecosystems such as freshwaters (Lake Michigan), the Hudson Estuary, and other smaller ponds and lakes all have a similar ^{137}Cs concentration factor relationship with organism size. This suggests that a mathematical framework may be constructed that incorporates the fundamental features of food chain transfer of a contaminant as well as physical uptake in exploring the observed concentration factors.

Simplified Model of Toxicant in Aquatic Food Chain

Although the distribution of a microcontaminant in the food chain can be described by using organism size as a continuous independent variable (Thomann 1979), the biological meaning of the coefficients in such a representation is not entirely clear. The discrete version of the continuous food chain model is a representation of compartment models of the food chain where a compartment is defined in specific biological terms. This latter approach is taken here in the development of a simplified model.

A substantial literature exists on compartment models for transfer of material through food chains and food webs and the related transfers of material. The ecological concepts of compartment analyses have been reviewed by Dale (1970) and Patten (1971) and examples of compartment models include Gillet (1974), Hill et al. (1976), Lassiter et al. (1976), Hoefner and Gillet (1976), and Aoyama et al. (1978). These approaches, however, tend to be somewhat complex in attempting to incorporate a variety of mechanisms or time-variable behavior. A great deal of flexibility is therefore realized in these models for detailed examination of specific problems. On the other hand, complexity sometimes obscures the basic relationships indicated by the preceding data. A simplified model is presented here that incorporates the basic features of the more complex model, but in a steady state condition for a linear food chain. To provide a first level of understanding of the mechanisms that are contributing to the observed concentration factors, a simple four-level food chain model can be considered. Figure 5 shows the schematic of the compartments:

1) Phytoplankton (less than 100 μm);
2) zooplankton (> 100 μm, < 300 mm);
3) small fish (approximately less than 300 mm);
4) large fish (top predator of size 0.1−1.0 m);

and mechanisms or processes in the model:

1) Sorption or direct uptake of the chemical from the water;
2) desorption and excretion of the chemical from the organism;
3) consumption of contaminated food;
4) respiration rate of organism.

Mechanisms (3) and (4) influence the weight change or growth of the organism as a function of age. The estimation of the available concentration of the contaminant in the water phase may be carried out through a separate model of the physical−chemical interactions of the particulate and dissolved components.

In the construction of the food chain model, a mass balance equation is written around each of the compartments incorporating the preceding mechanisms. The mass balance equation represents the flows of the chemical into and out of the average organism. Care must be taken to include the weight change of the organism since such changes affect the chemical concentration.

PHYTOPLANKTON

Consider the phytoplankton, detrital organic material, and other organisms, all of size <100 μm as the base of the food chain. An equation for this compartment is given by a simple reversible sorption−desorption linear equation as:

$$\frac{dv_1}{dt} = k_{u1}c - K_1 v_1 \tag{5}$$

where v_1 is the chemical concentration in the phytoplankton (μg/g(w)), k_{u1} is the uptake sorption rate (L/d-g(w)), K is the desorption and excretion rate (d^{-1}) and c is the concentration of the dissolved form of the chemical in water (μg/L).

GENERAL EQUATION FOR FEEDING COMPARTMENTS

For any food chain compartment that may accumulate the contaminant via ingestion as well as by sorption, a general mass balance equation for the linear food chain in Fig. 5 is

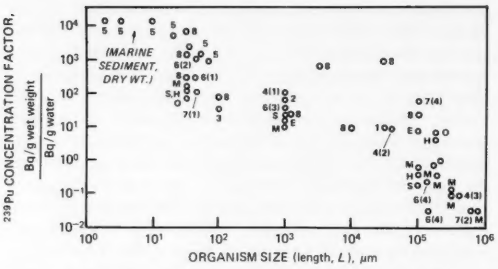

FIG. 3. Plutonium-239 bioaccumulation factors (wet weight basis) from field measurements. See Table 3 for key and references.

obtained as

$$(6)\quad \frac{dv''_i}{dt} = \frac{d(vw)_i}{dt} = \frac{w_i dv_i}{dt} + \frac{v_i dw_i}{dt} = k_{ui}w_i C - K_i v''_i + \alpha_{i,i-1}C_{i,i-1}v_{i-1}w_i \quad i = 2,3,4$$

where v''_i is the average chemical whole body burden (μg) and is given by

$$(7)\quad v''_i = v_i w_i$$

where v_i is chemical concentration in the ith compartment (μg/g(w)), w_i is the weight of the average individual (g(w)), k_{ui} and K_i are, as before, the uptake or sorption rate and desorption and excretion rate, respectively, for the ith organism, $\alpha_{i,i-1}$ is the chemical assimilation efficiency (μg chemical absorbed/μg chemical ingested), $C_{i,i-1}$ is the weight-specific consumption of organism i on $i-1$ (g(w) prey/g(w) pred.-d), and t is real time (days). The assimilation efficiency, α, represents that portion of the ingested contaminant mass that is absorbed and incorporated in organism tissue. The quantity $1 - \alpha$ then represents that portion of ingested chemical which is immediately or rapidly egested by the organism and never accumulated in body tissue. The excretion rate therefore in Eq. (6) is interpreted as excretion and desorption of the chemical that has been sorbed onto or into the organism.

An equation for the individual organism weight is

$$(8)\quad \frac{dw_i}{dt} = (a_{i,i-1}C_{i,i-1} - r_i)w_i, \quad i = 2,3,4$$

where $a_{i,i-1}$ is the biomass assimilation efficiency (g(w) Pred./g(w)prey) and r_i is the weight loss (d^{-1}) due to routine

TABLE 3. Explanation of symbols for Fig. 3. ^{239}Pu concentration factors, field observed.

Symbol	Location and organism	Reference
1	Cape Cod: mussel, clam	Noshkin (1972)
2	Ocean: zooplankton	Noshkin (1972)
3	Ocean: phytoplankton	Noshkin (1972)
	Coastal	
4(1)	Mixed zooplankton	Pillai et al. (1964)
4(2)	Mussels	
4(3)	Bonito (*Sanda sanda*)	
	Irish Sea	
5	Sediment	Hetherington (1978)
	Rocky Flats, Utah	
6(1)	Algae	Thompson (1975)
6(2)	Seston	
6(3)	Zooplankton and crayfish	
6(4)	Bullhead minnow (*Pimephales notatus*)	
	No. East Irish Sea	
7(1)	Algae	Hetherington et al. (1975)
7(2)	Plaice	
7(3)	Salmon	
7(4)	Mussels	
	Laurentian Great Lakes[a]	
S,M,H,E,O,$10^{1.5}$ μm	Phytoplankton and particulates	Marshall et al. (1975)
10^3 μm	Zooplankton	
10^5 to 10^6 μm	Alewife (*Alosa psuedoharengus*), coho, lake trout	
	"U" Pond	
8	A freshwater pond ecosystem, WA.	Emery and Klopfer (1977)

[a]S = Lake Superior, M = Lake Michigan, H = Lake Huron, E = Lake Erie, O = Lake Ontario.

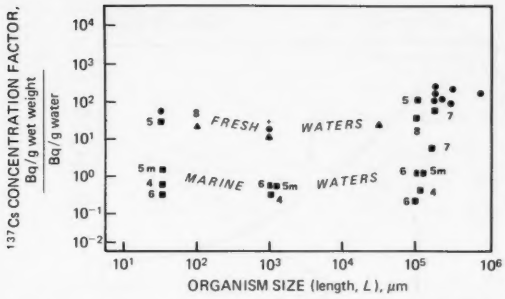

FIG. 4. Cesium-137 bioaccumulation factor (wet weight basis) from field measurements. See Table 4 for key and references.

TABLE 4. Explanation of symbols for Fig. 4. ^{137}Cs concentration factors, field observed.

Symbol	Location	Reference
0	Lake Michigan	Marshal et al. (1975)
Δ	Hudson Estuary	Mauro (1973)
+	Compilation of variety of water bodies	Vanderploeg et al. (1975)
4	Marine environment	Bowen et al. (1971)
5m	Marine environment	Jinks and Eisenbud (1972)
5	Fresh water bodies	Jinks and Eisenbud (1972)
6	Med. Sea, Israeli Coast	Gilat et al. (1975)
7	E. German Waters (fresh and marine)	Ettenhuber and Rohnsch (1975)
8	Par Pond, GA	Harvey (1964)

metabolism, swimming, and other activities. The weight change is therefore

$$(9) \qquad G_i = \frac{dw_i}{dt}/w_i = a_{i,i-1}C_{i,i-1} - r_i.$$

Equation (6) can therefore be written as

$$(10) \qquad \frac{dv_i}{dt} = k_{ui}c + \alpha_{i,i-1}C_{i,i-1}v_{i-1} - K'_i v_i, \qquad i = 2,3,4$$

where

$$(10a) \qquad K'_i = K_i + (dw_i/dt)/(w_i) = K_i + G_i.$$

Equations (10) and (10a) together with Eq. (8) and (9) form the basic mass balance chemical equations. The first term of Eq. (10) represents the direct uptake of the chemical by the organism from the water. The second term represents the flux of the chemical into the organism through feeding. Note that the units of this term are gram of chemical assimilated per gram predator per day. The third term is the loss of chemical due to desorption and excretion from body tissue at a rate K_i plus the change in concentration due to growth of the individual. This is reflective of the changing weight available through the size of the organism to "dilute" the concentration of the chemical. The equation pair, (9) and (10), can be solved analytically for the case of known food concentration and constant coefficients or numerically for time-variable coefficients. Norstrom et al. (1976) and Weininger (1978) are examples; in each case, however, the food toxicant concentration is assumed known. To obtain additional insight into the food chain transfer process, it is informative to consider a steady-state analysis of Eq. (10).

EQUILIBRIUM (STEADY STATE) CONDITION

The ith compartment around which the mass balance is taken to derive Eq. (6) is defined as a given age-class of an organism in the food chain. The properties of that age-class include the weight change as a result of net growth, as shown in Eq. (8). The net growth rate of the age-class can be considered to be a constant in time, i.e. that the average growth rate of a 2- to 5-yr-old top predator is the same from 1 year to the next. This assumption implies no temporal change in the feeding rate, food availability or respiration rate. Such an assumption may be entirely reasonable for the lower levels of the food chain but may be questionable for the top predators. Nevertheless, considerable insight can be gained into the behavior of the contaminant equations by assuming a constant growth rate. In a similar fashion, the assumption is made that the uptake, assimilation, and excretion rates are constant within a defined age-class (i.e. a defined compartment).

The solution to Eq. (9) for constant coefficients is

$$(11) \qquad w_i = w_{oi} \exp(G_i t)$$

where w_{oi} is the initial weight at the beginning of the age-class i.

The solution to Eq. (6), the body burden equation under an assumption of constant coefficients, is

$$(12) \qquad v''_i = \frac{(k_{ui}c + \alpha_{i,i-1}C_{i,i-1}v_{i-1})w_{oi}}{K'_i} (\exp(G_i t) - \exp(-K_i t)) + v''_{oi} \exp(-K_i t)$$

where v''_{oi} is the initial contaminant body burden at the beginning of the age-class i.

The solution to Eq. (10) is

$$(13) \qquad v_i = \frac{k_{ui}c + \alpha_{i,i-1}C_{i,i-1}v_{i-1}}{K'_i} (1 - \exp(-K'_i t)) + v_{oi} \exp(-K't).$$

Figure 6 shows the form of Eq. (11)–(13). The increase in the weight of the given age-class is exponential as shown in Fig. (6a), with a constant net growth rate of G_i. Figure (6b) shows the increase in body burden and indicates that no equilibrium is reached, i.e. the total mass of contaminant in the organism continues to grow as a result of uptake, assimilation, and increase in weight. Indeed, Eq. (12) shows that only if the net growth rate is zero (i.e. weight of the organism is a constant) is there an equilibrium condition on the body burden. A constant weight assumption, however, is a particularly poor one. Figure (6c) shows that even as weight is increasing exponentially and the body burden increasing according to Eq. (12), the concentration of the contaminant

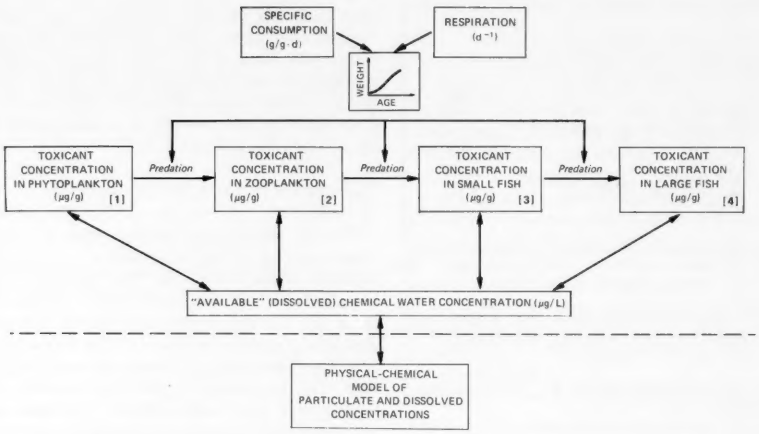

FIG. 5. Schematic of four-compartment food chain model.

approaches a constant value, an equilibrium or steady state condition. This equilibrium concentration represents a balance between the inputs of the contaminant to the organism and the mechanics of excretion and increasing weight of the organism. This can be seen from Eq. (10) where at equilibrium, $d\nu_i/dt = 0$, and

$$K'_i\nu_i = k_{ui}c + \alpha_{i,i-1}C_{i,i-1}\nu_{i-1}$$

or

$$(14) \qquad \nu_i = \frac{k_{ui}c + \alpha_{i,i-1}C_{i,i-1}\nu_{i-1}}{K'_i}$$

which can also be obtained from Eq. (13) at large t.

If then it is assumed that the growth and contaminant coefficients are constant over the length of time defined by the age-class of the compartment, then an equilibrium or steady state condition in the concentration of the contaminant is reached. This is the basis of considering an equilibrium or steady state model where the time derivative in Eq. (10) is set equal to zero.

One can also obtain from this line of reasoning the appropriate age-class interval for each compartment that will guarantee an approximate equilibrium condition. If a criteria of 90% of equilibrium is chosen, then the age interval t_a for a compartment is

$$(15) \qquad t_a \geq 2.3/K'.$$

For example, for a low excretion rate (e.g. $K = 0.001$/d) and a high net growth rate that is representative of the lower levels of the food chain (e.g. $G = 0.01$/d), $t_a \geq 209$ d. For a similar excretion rate but reduced net growth that is representative of the upper level of the food chain (e.g. $G = 0.001$/d) then $t_a \geq 3.1$ yr. Therefore if one considers the lower level to be representative of an age-class spanning about 0.5 yr and the upper food chain level to be representative of an age-class spanning about 3 yr, then an equilibrium, steady state concentration will be reached. Such a concentration will be approximately independent of the initial concentration at the beginning of the age-class as can be determined from Eq. (13). On the other hand, if excretion is very low, say approximately zero, and if the net weight change G is also very low, say approximately zero, then Eq. (15) indicates that the age span approaches infinity. This simply implies that an equilibrium condition will never be reached under those compartment characteristics. Such cases can occur for low excretion rates and older top predators, and therefore those situations are specifically excluded from the following steady state model. To summarize, equilibrium or steady state implies constant positive net weight change and constant uptake, assimilation, and excretion rates over an age-class interval defined to guarantee 90% of equilibrium. Each equation of the food chain can now be examined under these conditions.

Zooplankton — In Eq. (5) and (10) if $d\nu_i/dt = 0$, the steady state equation for the second (zooplankton) compartment is

$$(16) \qquad \nu_2 = \frac{k_{u2}c + \alpha_{21}C_{21}\nu_1}{K'_2}.$$

The concentration factor N_2 is then given from Eq. (2) as

$$(17) \qquad N_2 = N_{2w} + \frac{\alpha_{21}C_{21}}{K'_2}N_{1w}$$

where

$$(17a) \qquad N_{1w} = k_{u1}/K_1$$

and

$$(17b) \qquad N_{2w} = k_{u2}/K'_2.$$

Equations (17a) and (17b) represent the bioconcentration factors due to water uptake only. The total zooplankton bioaccumulation factor is thus composed of two parts: that due to direct sorptive uptake from the water and that due to predation

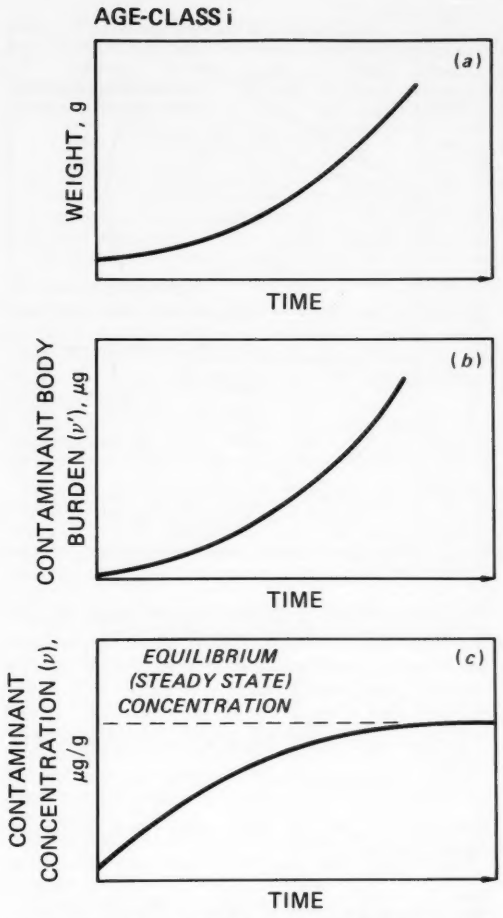

FIG. 6. Illustration of equilibrium conditions for concentration of contaminant in organism. (a) Exponential increase in organism weight, Eq. (11); (b) increase in contaminant body burden, Eq. (12); (c) increase in contaminant concentration to equilibrium (steady state) level, Eq. (13).

on contaminated phytoplankton.

Small fish — Following a development similar to the zooplankton, the steady state equation for the third compartment (small fish) is

$$(18) \quad \nu_3 = \frac{k_{u3}c + \alpha_{32}C_{32}\nu_2}{K'_3}.$$

Substituting Eq. (16) and solving for the small fish bioaccumulation factor gives

$$(19) \quad N_3 = N_{3w} + \frac{\alpha_{32}C_{32}}{K'_3}N_{2w} + \frac{\alpha_{32}C_{32}}{K'_3}\frac{\alpha_{21}C_{21}}{K'_2}N_{1w}$$

where $N_{3w} = k_{u3}/K'_3$, the bioconcentration factor for uptake of the chemical from the water only by the small fish. At this third level, the bioaccumulation factor is now seen to depend on the two lower levels through successive predation.

Large fish — For the top carnivore compartment, the procedure is now direct. The steady state toxicant concentration equation is

$$(20) \quad \nu_4 = \frac{k_{u4}c + \alpha_{43}C_{43}\nu_3}{K'_4}.$$

When successive substitutions are made for each of the lower trophic levels, the resulting bioaccumulation factor for the large fish is

$$(21) \quad N_4 = N_{4w} + \frac{\alpha_{43}C_{43}}{K'_4}N_{3w} + \frac{\alpha_{43}C_{43}}{K'_4}\frac{\alpha_{32}C_{32}}{K'_3}N_{2w} + \frac{\alpha_{43}C_{43}}{K'_4}\frac{\alpha_{32}C_{32}}{K'_3}\frac{\alpha_{21}C_{21}}{K'_2}N_{1w}$$

where $N_{4w} = K_{u4}/K'_4$. The additive effect of each of the lower three trophic levels on the top trophic level is indicated by this equation. It is not clear, of course, from this equation that there is a magnification of the contaminant from the lower to the upper trophic levels. Such a possibility depends on the ratio of the net assimilation of the chemical to the desorption and excretion and net weight change.

General Steady State Case and Further Simplification

The steady state bioaccumulation factor for the nth compartment in a linear food chain can then be written as

$$(22) \quad N_n = N_{nw} + \sum_{j=1}^{n-1}\left(\prod_{i=j+1}^{n} f_{i,i-1}\right)N_{jw}$$

where

$$(23) \quad f_{i,i-1} = \frac{\alpha_{i,i-1}C_{i,i-1}}{K'_i}.$$

The quantity f represents the potential relative increase or decrease in the bioaccumulation factor due to feeding on contaminated prey. Thus, f can be called the food chain transfer number. The quantity $\Pi f_{i,i-1}$ is the accumulated effect of the transfer through the $n-1$ levels and may be called the food chain multiplier, designated as Γ. The bioaccumulation factor therefore at any level of the food chain depends on several parameters which reflect the nature of the food chain and the properties of the chemical.

Consider now a further simplification. For convenience, let the food chain transfer number be a constant across the entire chain. Then

$$(24) \quad N_n = N_{nw} + \sum_{j=1}^{n-1} f^{n-j}N_{jw}$$

and the food chain multiplier Γ is simply

$$(25) \quad \Gamma = f^{n-j} = f^x$$

where $x = n - j$ is the number of levels in the food chain

below a given level of interest. Note also that $\log \Gamma = (n - j) \log f$ so that the food chain multiplier is linear in logarithms to the number of passes through the chain. Now it is clear that if $f < 1$, Γ decreases with increasing $n - j$. Figure 7 shows this result for $f = 0.5$ and 0.1. The decrease means that if the uptake due to feeding is less than the overall loss rate, then the upper levels of the food chain do not "feel" the chemical concentrations from compartments several levels below it. For this case the chemical concentration of the phytoplankton does not significantly influence the concentration of the chemical in the top predator.

Figure 7 also indicates that for $f > 1$, there can be a substantial magnification of the effect of food chain transfer. Values of f of about 2 to 3 can result in one to two orders of magnitude increase in the food chain magnification. The principal reason for this behavior can be seen by assuming that

$$(26) \quad (dw/dt)/w = a'C$$

where a' is an overall biomass conversion efficiency. Then the food chain transfer number is

$$(27) \quad f = \frac{\alpha C}{K + a'C}.$$

Now suppose $K \ll a' C$ (the case for substances such as PCBs with low excretion rates), then

$$(28) \quad f = \frac{\alpha}{a'}.$$

This equation simply states that the change in the concentration of the chemical due to predation on contaminated food is the ratio of the assimilation efficiency of the ingested chemical to the gross weight conversion.

Figure 7 therefore provides a theoretical basis for chemical magnification in the food chain. A food chain transfer number greater than one, which is representative of high chemical assimilation and low excretion, coupled with several passes through lower level compartments can result in increases of one or more orders of magnitude in the food chain multiplier. Conversely, if the transfer number is generally less than one, then the assimilation of the chemical may be low, excretion rate high, or a combination of both low assimilation and high excretion. The multiplier, Γ, then becomes progressively smaller than unity and the body burden of the top predator does not depend significantly on the lower levels of the food chain and is only dependent on direct uptake from the water.

Estimation of Parameters

The parameters for the above model are:

a) consumption, respiration, conversion efficiency, and growth rate;

b) uptake from water, excretion rate, and contaminant assimilation.

In principle, all parameters can be estimated independently of the field data through laboratory measurements.

For the first group, considerable information exists on respiration rates, rates of weight gain, and conversion efficiencies. All are of course species, age, and temperature-dependent functions of local habitat and associated food carrying capacity. A very approximate estimate of the growth,

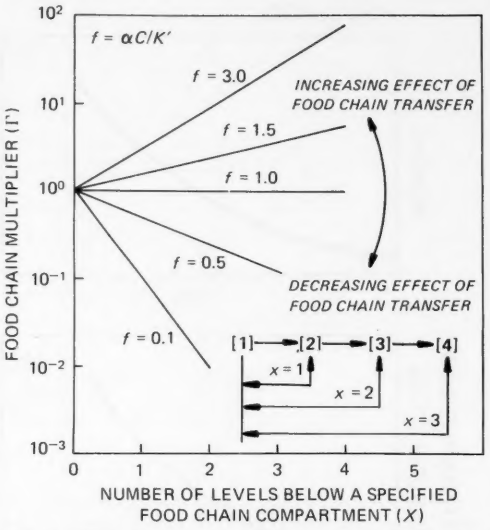

FIG. 7. Effect of constant food chain transfer number, f on food chain multiplier Γ.

Eq. (9), as a function of organism size, can be derived from the data compiled by Sheldon et al. (1973) as

$$G \simeq 14\, L^{-0.66} \text{ @ } 25°\text{C}$$
$$\simeq 4.2\, L^{-0.66} \text{ @ } 10°\text{C}$$

for L in μm and G in d^{-1}. For an allometric relationship of

$$L = 50\,000\, w^{0.33}$$

with w in g(w), the net weight change as a function of organism weight is

$$(29a) \quad G \simeq 0.01\, w^{-0.22} \text{ (@ } 25°\text{C)}$$
$$(29b) \quad \simeq 0.002\, w^{-0.22} \text{ (@ } 10°\text{C)}.$$

G can also be estimated from $(dw/dt)w$, if information is available on the weight of the organism as a function of age. With an estimate of the net weight change, C the average food consumption can be estimated from Eq. (9) if the respiration and conversion efficiency are known and assumed constant, i.e.

$$(30) \quad C_{i,i-1} = \frac{G_i + r_i}{a_{i,i-1}}.$$

A variety of data exist on the respiration rate of aquatic organisms and the relationship of respiration to body weight. Norstrom et al. (1976) discuss and summarize much of the available literature. For routine metabolism,

$$(31a) \quad r \simeq 0.036\, w^{-0.2} \text{ @ } 25°\text{C}$$
$$(31b) \quad \simeq 0.014\, w^{-0.2} \text{ @ } 10°\text{C}$$

for r in d^{-1}. The total metabolic rate also includes energy

TABLE 5. Assumed growth and feeding parameters.

Trophic level	Assumed range of wt-(g(w))	G, net wt change (1/d)[a]	r, wt loss rate (1/d)[b]	a, food conversion eff. (g/g)	Food consumption (g/g-d)[c]
2	0.001−1	0.0092	0.075	0.8	0.105
3	0.05−50	0.0048	0.0087	0.8	0.017
4	5−5000	0.0018	0.0055	0.8	0.009

[a]Assigned from ranges computed from Eq. (29a) and (29b).
[b]Assigned from ranges computed from Eq. (31a) and (31b).
[c]Calculated from Eq. (30).

expenditures for growth-related activities such as foraging, and Norstrom et al. (1976) have discussed the further expansion of Eq. (31) to incorporate these metabolic functions. For the overall purposes here however, Eq. (31) serve as a useful first approximation.

The conversion efficiency, a, is also species and temperature-dependent but for this work, a constant value of 0.8 is assigned following Norstrom et al. (1976).

For those parameters that are specific to the chemical, laboratory measurements can be obtained of uptake, excretion, and chemical assimilation rates. For most purposes, however, experimental data are sparse for the top predators, especially adult fish and for the intermediate levels of the food chain.

Some attempts can also be made to relate these chemical parameters to more fundamental quantities such as water solubility or octanol−water partition coefficients (Kenaga and Goring 1978; Neeley et al 1974). Such attempts, however, specific to fish and not the lower levels of the food chain, are for water uptake only and the ratio k_u/K rather than the individual parameter. (As can be seen from the model equations, it is necessary to separate k_u and K and only if clearance experiments are carried out can the individual parameters be estimated.)

Measurements of the excretion rate have been carried out for a variety of substances and have been summarized by Thomann (1978).

It appears that little fundamental work has been carried out relative to the assimilation of contemporary chemicals from ingested food, although data do exist for some substances such as the radionuclides and heavy metals.

The procedure for model calibration then is to estimate the food chain feeding parameters from the known relationships in the literature. For a given chemical such as PCBs, estimate the uptake and excretion and assimilation ratio. With the estimated parameters, calculate the food chain response with the above model, using PCB as a calibrating variable.

Following a similar procedure and without changing the food chain feeding parameters, calculate the ^{239}Pu and ^{137}Cs distribution using estimates of uptake, excretion, and assimilation.

Model Calibration: PCB, ^{239}Pu, and ^{137}Cs

The simple steady state model of Eq. (21) can now be applied to the bioaccumulation data on PCB, ^{239}Pu, and ^{137}Cs discussed earlier. The first step is to choose a representative set of parameters for the food chain shown in Fig. 5.

TABLE 6. Assumed PCB uptake, excretion, and assimilation efficiency parameters.

Food chain level	PCB (K = O) k_u[a]	K[b]	N'_w[c]	α[d]	PCB ($K \neq$ O) k_u	K	N'_w	α
1	—	—	$10^{5.50}$	—	—	—	$10^{5.50}$	—
2	753	0	$10^{5.60}$	0.9	753	0.01	$10^{5.29}$	0.9
3	86	0	$10^{4.85}$	0.9	86	0.004	$10^{4.59}$	0.9
4	55	0	$10^{5.09}$	0.9	55	0.001	$10^{4.90}$	0.9

[a]Uptake rate (g/g(w)−d).
[b]Excretion rate (1/d).
[c]Bioconcentration from water only (g/g(d)÷g/g) = k_u/K'.
[d]Assimilation efficiency of microcontaminant.

Food Chain Parameters

Following the estimating equation for net weight change, respiration, and food consumption, a range of weights was assigned to each feeding compartment. For this range and the temperature range from 10 to 25°C, values for G and r were assigned. For a fixed conversion efficiency of 0.8, the average food consumption was calculated from Eq. (30). The assigned and computed values are shown in Table 5. These are obviously not uniquely determined values but are meant to be broadly representative of organism behavior at the lower, intermediate, and upper levels of the food chain.

The choices shown in Table 5 represent a food chain typical of phytoplankton, detritus —> large crustacean —> small fish (300 mm) —> top predator.

Examples of large crustaceans would be *Mysis relicta* as in Lake Michigan or large *Neomysis mercedis* (>10 mm) in the Sacramento−San Joaquin Delta. The crustaceans feed directly on detritus and phytoplankton and therefore form an important first link in the food chain. The small fish would represent juveniles and adults of a wide variety of fish species including alewife, perch, anchovy, and menhaden. The top predator of up to 5 kg weight is representative of a 2- to 5-yr-old striped bass or lake trout.

PCB Calibration

After assignment of the growth and feeding parameters, the PCB data shown in Fig. 1 are used to calibrate the model. Adjustments were made in the various coefficients to calibrate to the observed data, but such adjustments were always maintained within reported ranges of the coefficients. The resulting PCB specific parameters are shown in Table 6. Two cases were considered: first, a calculation assuming that the clearance of PCB is approximately zero (a case that bounds the results but also admits of some realism based on laboratory experiments by Defoe et al. 1978); secondly, a calculation assuming excretion rates as a function of size following the approximate trend for PCB given by Thomann (1978). The values for N'_{iw}, the bioconcentration factor due to water uptake only are representative of the data shown in Fig. 1. The value of α at 0.9 has not been checked and is set at this high value because PCBs are lipid-soluble. The results of applying the model using the parameters of Tables 5 and 6 are shown in Fig. 8.

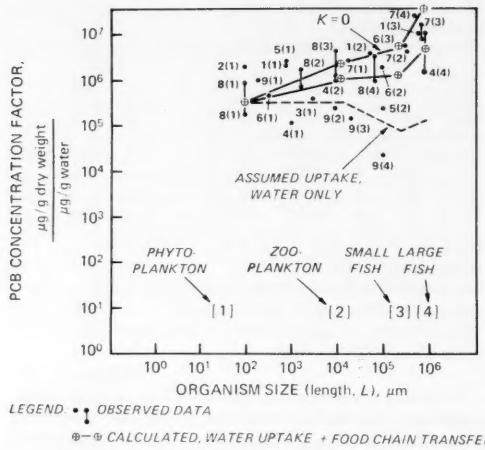

FIG. 8. Comparison of field-observed PCB concentration factors and simple food chain model calculation.

Several important points can be noted. The model calculation brackets the observed data in the upper trophic levels and hence may represent a meaningful representation of the mechanisms giving rise to the observations. In addition, for the top predator, the results in Fig. 8 for level 4 are consistent with the data shown in Fig. 2 for the Hudson Estuary. The dry weight bioaccumulation factors shown in Fig. 8 (range of N' from $10^{6.0}$ to $10^{7.4}$) are one to two orders of magnitude greater than the concentration factors used for uptake of PCBs from water only as shown in Table 6 (range of N'_{iw} from $10^{4.6}$ to $10^{5.6}$). The differences are due to food chain accumulation and are most significant at the top predator level. The decrease of N' at the top level from $10^{7.4}$ to $10^{6.6}$ reflects the inclusion of nonzero excretion rates which reduced the concentration factor by about an order of magnitude. This model indicates that for PCB, the observed concentration in the top predator fish is almost entirely due to food chain transfer and not uptake directly from the water. This result casts doubt on the adequacy of empirical relationships for PCB (or similar substances) that estimate concentration factors from laboratory experiments involving uptake of the chemical from water only. Inferences drawn from such experiments and relationships as to the potential effect on the aquatic ecosystem or human health could be in error by more than one order of magnitude.

^{239}Pu Calibration

The data on bioconcentration of ^{239}Pu directly from water are somewhat sparse. Experiments with mussels and shrimp by Fowler et al. (1975) indicated $N = 2-20$, significantly below the values for PCB. Uptake experiments of ^{241}Am by algae yielded bioconcentration factors of $10^3 - 10^4$ (Gisey and Paine 1977). Fowler et al. (1975) also determined excretion rates of plutonium of 0.0009 to 0.016 d^{-1} for the mussel and shrimp, respectively. A dependence of excretion rate on body weight of mammals has also been summarized (ICRP 1972). The reported range for mammalian livers is from 0.0006 to 0.02 d^{-1} in the 10- to 10^4-g weight interval. Values for the excretion of plutonium from the skeleton are one half to one order of magnitude less than the liver values. Data available on absorption of plutonium from the gastrointestinal tract for mammals (ICRP 1972) indicate that the values are very low and generally less than 1%. Fowler and Guary (1977), however, measured absorption efficiencies of 20–60% in crabs and suggested that the data from mammals may not be applicable to marine invertebrates. The results of the model calibration given below, on the other hand, tend to support a low absorption efficiency in aquatic food chains.

Table 7 indicates the parameter choices for ^{239}Pu. The results of the model calculation using these values are shown in Fig. 8. As shown, the calculated four orders of magnitude decrease in the bioaccumulation factor is representative of the observed data and results principally from the assumed low absorption efficiency and the excretion rates shown in Table 7. If the higher levels of Fowler and Guary (1977) are used, the calculated decline is only about one order of magnitude for the same excretion rates. The calculations compare well to the observed data at the higher absorption efficiency only if the levels of excretion are increased to values greater than 2 per day. Such excretion levels, however, appear unjustified based on the measurements of Fowler et al. (1975). One tentatively concludes therefore that the observed ^{239}Pu concentration factor behavior is due principally to low absorption of ^{239}Pu from food, and associated levels of excretion of 0.01–0.05 per

TABLE 7. Assumed uptake, excretion, and absorption efficiency parameters for ^{239}Pu and ^{137}Cs.

Food chain level	^{239}Pu				^{137}Cs					
					Freshwater		Marine			
	k_u[a]	K[b]	N_w[c]	α[d]	k_u	N_w	k_u	N_w	K	α
1	—	—	10^4	—	—	10^3	—	$10^{1.5}$	—	—
2	18.7	0.05	$10^{2.5}$	0.01	4.9	10^2	0.49	10^1	0.03	0.5
3	0.3	0.02	10^1	0.01	0.4	$10^{1.5}$	0.07	$10^{0.75}$	0.003	0.5
4	0.01	0.01	10^0	0.01	0.04	10^1	0.01	$10^{0.5}$	0.0018	0.5

[a]Uptake rate (g/g(w)–d).
[b]Excretion rate (1/d).
[c]Bioconcentration from water only (Bq/g(w)÷Bq/g) = k_u/K'.
[d]Assimilation efficiency of microcontaminant.

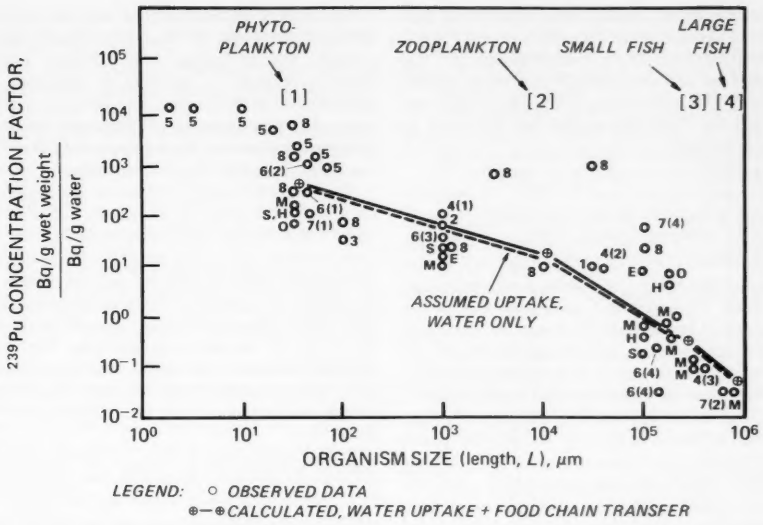

FIG. 9. Comparison of field-observed ^{239}Pu concentration factors and simple food chain model calculation.

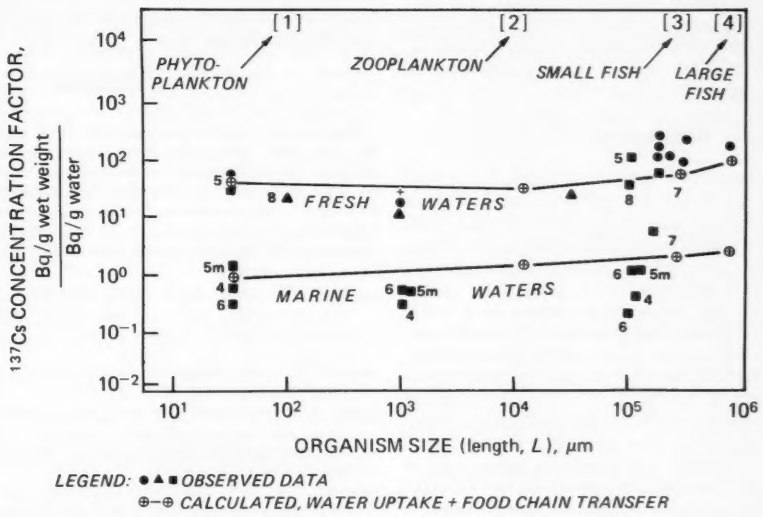

FIG. 10. Comparison of field-observed ^{137}Cs concentration factors and simple food chain model calculation.

day. Figure 9 also indicates that as a consequence, the difference between the concentration factor due to water and food and that due to water alone is insignificant. A reasonable estimate then of ^{239}Pu body burden can be obtained by a simple partitioning model that relates water concentration to body burden directly without any effect of food chain transfer.

^{137}Cs Calibration

Reported bioconcentration factors for ^{137}Cs uptake by fish and mollusks from water only range from 1 to 100 depending on organism and test conditions (Bhatt 1966; Davis 1961; Mauro 1973; Harrison 1973). Laboratory values for biocon-

centration factors for algae parallel those of the field data (range 1−100) and depend on salinity (Harvey and Patrick 1967; Gutknecht 1964; Rice 1961). Excretion data of ^{137}Cs have been summarized by Vanderploeg et al. (1975), Mauro (1973), and others. The data show a strong dependence on organism size and the following relationship was used to estimate the excretion rate:

$$(27) \quad K = 10\,L^{-0.625}$$

for L in μm and K in d^{-1}. Hewett and Jeffries (1978) measured absorption of ^{137}Cs from food at 42% for plaice and 67% for trout, and Mauro (1973) has summarized efficiencies for several species of fish feeding on various food sources and reports a range from 3% for bluegill on detritus to 92% for bluegill on chironomid larvae; Table 7 shows the choice of parameters for uptake, excretion, and absorption. Two levels of bioconcentration factors for uptake directly from the water were chosen to represent the freshwater and marine cases.

The results of the calculation and the comparison to the observed field data are shown in Fig. 10. The increase in the bioaccumulation factor is not marked from the phytoplankton to the top predator in either case, although for both cases, the ^{137}Cs body burden at the top of the food chain is due principally to the food route. The calculation indicates that the initial salinity-dependent sorption by the phytoplankton dominate the result at the top predator level. For the freshwater case, 91% of the calculated ^{137}Cs accumulation factor at the fourth level is due to the phytoplankton and for the marine case, 70% is due to the phytoplankton. The ^{137}Cs case therefore is of interest since it represents an example of intermediate accumulation. the level of which is strongly dependent on phytoplankton sorption.

Conclusions

It is concluded from this work that a simple steady state model of transfer of chemicals in the food chain can aid in explaining the observed concentration factors for three substances that exhibit widely varying behavior in the food chain. The simple steady state model indicates that the parameters of food assimilation, excretion, and net weight gain are important characteristics that determine the degree to which a chemical bioaccumulates in the food chain. For a substance such as PCB, the data and the model analyses indicate that at the top predator level, almost all of the observed body burden can be attributed to the transfer of PCBs from contaminated food. The bioaccumulation is due to the postulated relatively high adsorption of the PCB from the contaminated food and the relatively low excretion of PCB from the upper levels of the aquatic ecosystem. Some exceptions in specific food chains, however, do exist. For a substance such as ^{239}Pu, the data and model analyses indicate that the effect of food chain transfer is insignificant and the observed body burden, in the upper level of the food chain, is due almost entirely to direct adsorption from the water. The application of the analysis to ^{137}Cs indicates concentration factors that are at an intermediate level between PCB and ^{239}Pu. The observed cesium concentration factors are due principally to food chain transfer with a high dependence on the salinity-dependent phytoplankton adsorption.

The analysis of data, coupled with the steady state model, indicates that a specific microcontaminant behaves in a surprisingly similar manner across diverse food chains. The analysis, however, is an order of magnitude, analysis and individual food chains may vary from the average, although across the entire spectrum of freshwater, lake, estuarine, and open marine systems, the behavior of PCB, ^{239}Pu, and ^{137}Cs can be explained by similar principles.

The simplified model of chemical accumulation in the food chain can, therefore, be of specific value in assessing the relative effect of the food route versus the water route in the chemical body burden for the upper levels of the food chain. The analysis indicates that for substances such as PCBs, the actual field concentration in the top predator may be at least one order of magnitude higher than presently assumed from the results of laboratory uptake data. The framework also indicates the need to obtain information on chemical absorption efficiencies and excretion rates especially for the top predators and also for the intermediate levels of the food chain.

To first approximation then, the accumulation of chemicals in the food chain may, through the use of a steady state model, be calculated and forms a basis for estimating the allowable discharge of a substance so as not to exceed a given concentration in the food chain. The food chain transfer number and the food chain multiplier are simple approximations to a complex system but illustrate the basic approach that can be used in assessing the potential buildup of a chemical in a food chain. For any specific food chain, however, more detailed compartment analyses must be carried out.

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Restricted Least-Squares Estimates of Age Composition from Length Composition

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CLARK, W. G. 1981. Restricted least-squares estimates of age composition from length composition. Can J. Fish. Aquat. Sci. 38: 297–307.

An exploited stock's length distribution over a set of fixed length intervals can be represented as the product of its age distribution vector and a matrix whose columns are the length distributions of the various age-groups. This matrix should be reasonably stable over time, so it can be estimated from age–length sampling done at any time. Then, so long as the number of length intervals exceeds the number of age-groups, a restricted least-squares estimate of the age distribution underlying any sample length distribution can be found with certainty by quadratic programming. For practical work, the advantages of this method over numerical and closed-form alternatives are that the estimates are reproducible and never contain any negative values. A general statistical treatment is impossible owing to the exclusion of negative values and the presence of sampling error in the matrix of estimated age-specific length distributions, but simulation trials show that in commercial fishery assessments least-squares estimates can be obtained that are unbiased and nearly as precise as maximum-likelihood estimates.

Key words: age composition, age–length key, linear regression, restricted least-squares, quadratic programming, simulation

CLARK, W. G. 1981. Restricted least-squares estimates of age composition from length composition. Can. J. Fish. Aquat. Sci. 38: 297–307.

La distribution des longueurs d'un stock exploité, sur une série d'intervalles de longueur fixes, peut être représentée comme le produit du vecteur distribution des âges et d'une matrice dont les colonnes sont les distribution des longueurs des divers groupes d'âge. Cette matrice devrait être raisonnablement stable dans le temps, de sorte que l'on peut l'estimer à partir d'un échantillonnage des âges et des longueurs effectué en aucun temps. On peut ensuite trouver avec certitude, à l'aide programmation quadratique, une estimation par moindres carrés limités de la distribution des âges correspondant à celle des longueurs d'un échantillon quelconque, pourvu que le nombre d'intervalles de longueur dépasse celui des groupes d'âge. Dans la pratique, cette méthode a sur les méthodes numériques et autres les avantages suivants : les estimations sont reproductibles et elles ne contiennent jamais de valeurs négatives. Il est impossible de procéder à un traitement statistique général par suite de l'exclusion de valeurs négatives et de la présence d'erreurs d'échantillonnage dans la matrice de distribution estimée des longueurs spécifiques à l'âge. Par contre, des simulations démontrent que dans les évaluations de pêches commerciales, on peut obtenir des estimations par moindres carrés qui ne sont pas biaisées et qui sont presque aussi précises que les estimations de probabilité maximale.

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THE age composition of commercial catches is usually estimated by applying an age–length key to the length distribution (Ketchen 1950). There are two kinds of sampling involved in the procedure: measuring a large sample to estimate the total length distribution over a set of fixed length intervals, and aging a relatively small sample from each length interval to estimate the age distribution therein. If both kinds of sampling are done at the same time, the procedure gives unbiased estimates of the total age distribution at that time.

It frequently happens, however, that no age subsampling is done in conjunction with the length sampling of catches from a stock in some period. Later, when the age composition in that period is needed for an assessment, the common practice is to apply an age–length key from some other period. But as mentioned by Kimura (1977) and later explained in detail by Westrheim and Ricker (1978), this expedient will almost always give biased estimates when there is substantial overlap between the length distributions of successive age-groups, as there usually is. The cause of the bias is that the age distribution within each length interval, which is the basis of an

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age–length key, depends on the relative abundances of the age-groups represented there, and hence on the age distribution of the sampled population as a whole. The effect is that an age–length key will consistently produce an estimated age distribution very similar to the one underlying the key, regardless of the age distribution underlying any length sample to which the key is applied. Thus the common practice of applying age–length keys promiscuously is at best pointless, and can be dangerous when, as it will, the procedure masks an important change in age composition.

Accurate estimates of age composition can be obtained from mismatched length samples and age–length sampling data by methods developed to decompose what are usually called finite distribution mixtures (Macdonald 1975). Whereas the age–length key is based on age distributions within length intervals, distribution mixture methods are based on length distributions within age-groups, which do not depend on the age composition of the stock as a whole and therefore can be estimated from a sparse set of age–length samples and applied to a full set of length samples.

In fisheries, the problem of decomposing a total length distribution into its component age-groups has been treated in three ways:

i) Graphical techniques (e.g. Cassie 1954). These differ from the numerical methods discussed below only in being necessarily subjective and limited. They will not be considered here.

ii) Numerical methods (Hasselblad 1966; Kumar and Adams 1977). These were developed to estimate not only the relative age frequencies but also some or all of the parameters of the component distributions, assumed to be normal. Such methods therefore do not require independent estimates of the component distributions, but can make use of whatever estimates are available to simplify the numerical search for the remaining estimates and increase their precision. If all of the component distributions can be specified beforehand, these methods reduce to a numerical search for estimates (usually maximum-likelihood estimates) of only the relative age frequencies. The search can be restricted so that the estimates will all be nonnegative and will sum to one, as realism requires, but for purely computational reasons the search cannot be guaranteed to locate the desired estimates very precisely.

iii) Multiple regression (Macdonald 1969, 1975). An alternative when estimates of all the component distributions are available is to obtain least-squares or similar estimates of the relative age frequencies by regression. This method guarantees exact estimates, which can be required to sum to one, but some of them may be negative.

The method of Macdonald and Pitcher (1979) is a hybrid in which relative age frequencies are estimated by regression during each iteration of a numerical search for estimates of any unknown parameters of the component length distributions, whose form is assumed known. If all the component distributions are specified in advance, either parametrically or empirically, the method reduces to one multiple regression.

This paper describes a quadratic programming procedure that requires estimates of all the component length distributions and guarantees exact, nonnegative least-squares estimates of the relative age frequencies. While no general statement can be made about the bias and variance of these estimates, simulation trials reported below indicate that with sample sizes typical in commercial fisheries, they will not be biased and will be nearly as precise as maximum-likelihood estimates.

Formulation of the Problem

Consider a stock with a certain schedule of growth in length with age. This schedule is usually described by the mean length at each age, but another characteristic of it is the distribution of lengths about the mean at each age.

To be precise, let x_{ij} denote the proportion of animals of age j that fall within length interval i. Similarly, let π_j denote the proportion of the total population that belongs to age-group j, and p_i the proportion of the total population that falls within length interval i. Then clearly

$$p_i = \sum_j x_{ij}\pi_j.$$

In matrix notation, this summation can be expressed conveniently for all age-groups and length intervals as

$$\mathbf{X}\boldsymbol{\pi} = \mathbf{p}$$

where $\boldsymbol{\pi}$ and $\mathbf{p}$ are column vectors representing the relative distributions of age and length, respectively, within the population as a whole, and $\mathbf{X}$ is a matrix each of whose columns represents the relative length distribution of a particular age-group. Thus the length distribution of the exploited stock is obtained as the weighted sum of the length distributions of the component age-groups, the weights naturally being the relative frequencies of the age-groups. This is the basic formulation of the age–length problem as a distribution mixture problem.

For any exploited population at any time, the linear model $\mathbf{X}\boldsymbol{\pi} = \mathbf{p}$ is an identity. Over time the age distribution $\boldsymbol{\pi}$ may vary widely with changes in recruitment and mortality, but the matrix $\mathbf{X}$ will not vary much unless there are considerable changes in the growth schedule or, possibly, in the level or pattern of fishing mortality where it is size selective. Barring large changes of this sort, the age and length compositions of an exploited stock, however, much they vary over time, will always be related by the system of linear equations $\mathbf{X}\boldsymbol{\pi} = \mathbf{p}$, with the matrix of coefficients $\mathbf{X}$ practically constant.

This is the case that will be considered here. Obviously if the matrix $\mathbf{X}$ of age-specific length distributions is constant, it can be estimated from age–length sampling done at any time, and the estimate can then be used to infer the age distribution corresponding to any length distribution by solving the system of linear equations in one of several possible ways.

Suppose the data for a given stock include I length intervals and J age-groups. Then superficially the model is a system of I equations (one for each length interval) in J unknowns (the relative frequencies of the age-groups), but in fact there are only I-1 equations and J-1 unknowns because each of the relative frequency distributions must sum to one. The practical problem is how to estimate $\boldsymbol{\pi}$ given sample estimates of

$\mathbf{X}$ and $\mathbf{p}$. If $I < J$ (i.e. the number of length intervals is less than the number of age-groups with distinct length distributions), there will usually be a multiplicity of algebraic solutions and therefore no useful estimates. If $I > J$, the system will usually not have an algebraic solution, but solutions can in theory be obtained to satisfy any of several of fitting criteria, such as maximum likelihood, minimum chi-squared, and the various forms of least squares. In the special case $I = J$, there will be exactly one algebraic solution, and this solution will necessarily satisfy all of the usual fitting criteria.

However obtained, the estimated age distribution $\hat{\pi}$ must satisfy two conditions to be useful in practice:

i) the elements must sum to one:

$$\sum_j \hat{\pi}_j = 1$$

ii) all of the elements must be nonnegative:

$$\hat{\pi}_j \geq 0, j = 1, \ldots, J.$$

Throughout this paper, estimates forced to sum to one will be called constrained, and estimates forced to be nonnegative as well will be called restricted. The unit-sum constraint is linear and can be satisfied with little difficulty, but the nonnegativity condition is nonlinear, and it will not in general be satisfied by the solutions computed by standard methods with sample data to meet any of the various fitting criteria. For example, the unrestricted unit-sum maximum-likelihood or ordinary least-squares solution can include negative elements, which will not be useful in stock assessments.

In what follows, the problem of computing restricted estimates will first be treated for the case where the length distribution matrix $\mathbf{X}$ is known exactly, and then the effect of estimating $\mathbf{X}$ will be considered.

Estimation with a Known Length Distribution Matrix

Suppose that $\mathbf{X}$ is known exactly, that $I > J$, and that the age composition corresponding to a sample length distribution $\hat{\mathbf{p}}$ (drawn from a multinomial distribution with true proportions $\mathbf{p}$) is to be estimated according to some fitting criterion. Macdonald and Pitcher (1979) summarize and compare the various criteria, some of them equivalent, that have been adopted. Every criterion is essentially some measure of distance between the sample length distribution $\hat{\mathbf{p}}$ and the estimated true length distribution $\tilde{\mathbf{p}}$ computed from an estimated age distribution $\hat{\boldsymbol{\pi}}$, or $\tilde{\mathbf{p}} = \mathbf{X}\hat{\boldsymbol{\pi}}$.

For the purposes of this paper, all the criteria can be divided into two groups:

i) the various least-squares criteria, including:

$$Q_0 = \sum_i (\hat{p}_i - \tilde{p}_i)^2 \quad \text{ordinary least squares}$$

$$Q_1 = \sum_i w_i (\hat{p}_i - \tilde{p}_i)^2 \quad \text{weighted least squares where the weights do not depend on either the sample or calculated length distribution}$$

$$Q_2 = \sum_i (\hat{p}_i - \tilde{p}_i)^2/\hat{p}_i \quad \text{modified minimum chi-squared, which is weighted least squares in which the sample length distribution determines the weights}$$

ii) all other criteria, including

$$Q_3 = \sum_i \hat{p}_i \ \ell n \, \tilde{p}_i \quad \text{maximum likelihood}$$

$$Q_4 = \sum_i (\hat{p}_i - \tilde{p}_i)^2/\tilde{p}_i \quad \text{minimum chi-squared.}$$

All these distance measures are concave functions (up or down) of the age distribution $\hat{\boldsymbol{\pi}}$ (considered here as a variable), and since the set of all unit-sum, nonnegative age distributions is convex, the existence of a unique restricted estimate is assured in every case (Simmons 1975). The important difference between the least-squares criteria and the others is that constrained least-squares estimates can be computed in closed form by multiple regression, while maximum-likelihood and minimum chi-squared estimates of any kind require some sort of numerical search.

Hasselblad (1966) and Kumar and Adams (1977) have described various search algorithms for locating restricted maximum-likelihood estimates. These are iterative procedures that move from point to point, steadily reducing the distance between the current estimate and the maximum-likelihood estimate, whose position is unknown. When only the relative age frequencies are being estimated, any of these algorithms can be guaranteed to locate an estimate with an error less than any arbitrarily prescribed level if a sufficient number of iterations is allowed, but there is no way to know the relationship between the number of iterations and the remaining error in any given application. In practice, therefore, every algorithm has a convergence criterion that causes it to halt whenever the rate of change of the current estimate or the likelihood function falls below some prescribed value. This rule produces good estimates if the likelihood function is sufficiently sensitive to every element of the estimated age distribution, but when some elements are poorly distinguished from their neighbors by closely corresponding columns in the matrix $\mathbf{X}$ the likelihood function will be relatively insensitive to them and a search algorithm will be likely to halt some distance away from the maximum-likelihood values of those elements. This result is usually called false convergence, and when the maximum likelihood values are in fact small, the relative errors can be large. Even such robust techniques as the Nelder–Mead algorithm are subject to errors of this sort (Nelder and Mead 1965). The upshot is that despite the theoretical possibility of finding the restricted maximum-likelihood estimate (or any of the others) by means of a restricted numerical search, in practice one can never be sure how far from the desired point a search algorithm halted. As a result, the properties of estimates obtained in this way cannot be stated with certainty, and their values in a particular application can vary considerably according to the search algorithm and convergence criterion used.

Macdonald (1969, 1975) and Macdonald and Pitcher (1979) have explained how to compute constrained least-

squares estimates, but as mentioned above these will not in general be nonnegative. The unit-sum age distribution that best fits a sample length distribution may involve weighted differences as well as weighted sums of the columns of **X**, so the constrained estimates may include some negative elements. In simulated tests conducted by the author, this outcome was the rule rather than the exception when the matrix **X** included some close columns representing the similar length distributions of older age-groups.

If the constrained estimate has no negative elements, it is also the restricted estimate. Otherwise the restricted estimate will be different and will necessarily have a larger sum of squares, but it will be the best sensible estimate (i.e. the set of nonnegative relative age frequencies that best fits the sample length distribution according to the criterion adopted).

The restricted estimate cannot be computed in closed form, but for any of the least-squares criteria it can be found with certainty by quadratic programming. In mathematical programming terms, all the least-squares criteria are quadratic objective functions, so the unique minimum in every case can be found by a simplex algorithm (Simmons 1975). Choi and Bulgren (1968) remarked that restricted estimates could be obtained by quadratic programming if needed, but apparently did not choose to do so in their own work.

The logic of the procedure can be explained briefly. The object is to find that point in the feasible region (consisting of all unit-sum, nonnegative age distributions) where the objective function is minimized. If the constrained estimate is itself nonnegative, then that is the point. If the constrained estimate lies outside the feasible region (i.e. has some negative elements), it follows from the form of the objective function that the restricted estimate must lie on a boundary of the feasible region, where some of the elements are zero. As it affects fitting, setting an element to zero is equivalent to deleting it from the parameter vector and deleting the corresponding column from the regression matrix,thus reducing the model. Minimizing values of the remaining, free elements can then be computed in closed form as the constrained estimate for the reduced model. One way to find the restricted estimate would therefore be to compute the constrained estimate for every possible reduced model and choose the nonnegative one that produced the smallest value of the objective function (Waterman 1974), but this procedure is prohibitively arduous when more than a few age-groups are involved. Starting from any point in the feasible region, a simplex algorithm will locate the same point by following the descent of the objective function along a broken path on the boundary, through a sequence of increasingly favorable reduced models, until locating the point that satisfies the Kuhn–Tucker conditions, and that is therefore the desired estimate. The algorithm is guaranteed to reach this point within a finite number of iterations because each iteration consists of fitting a previously untried reduced model, and there is only a finite number that can possibly be tried. Moreover, the point located will be the exact restricted least-squares estimate, since every element will be either exactly zero or one of the exact values computed in closed form for the best reduced model.

As a practical matter, the unit-sum and nonnegativity conditions imposed on the estimate translate into very simple Kuhn–Tucker conditions that in turn permit a fairly simple computer version of the simplex algorithm (available in ANSI FORTRAN from the author). This routine is also fast in comparison with search methods. On a CDC CYBER 73 computer, estimating an age composition with seven age-groups and 14 length-groups required less than 1 s of central processor time on average. This problem involved $2^7 - 1 = 127$ possible reduced models, but the solution never required more than five iterations (i.e. five constrained regressions) in several thousand trials.

Calculating exact restricted estimates to satisfy any of the least-squares criteria is therefore not a problem. The problem is to determine how these estimates will behave, since the nonnegativity condition precludes a general treatment of their distributional properties. In particular, no general statement can be made about their bias and variance.

If an analogy with the corresponding constrained estimates is valid, the most precise of the three forms of least-squares estimates should be the modified minimum chi-squared (MMCS) estimate, since it is asymptotically equal to the maximum-likelihood estimate (Macdonald and Pitcher 1979), and at least in commercial fisheries assessments the length samples are usually large, comprising a few hundred to a few thousand animals. Ordinary least-squares (OLS) estimates should be the least precise, and weighted least squares should be intermediate so long as fixed weights can be prescribed that are independent of the length sample but correspond more closely to the reciprocals of the true relative length frequencies (**p**) than do equal weights. (Length frequencies calculated from an arbitrary age distribution could be expected to provide such weights.)

Although the most promising, MMCS estimates do have some disadvantages. Since the reciprocals of the sample relative length frequencies are used as weights, sample frequencies of zero are impermissible, although they can and do occur in practice. When they do, MMCS estimation requires that they be deleted from the vector of observations $\hat{\mathbf{p}}$, the corresponding rows be deleted from the length distribution matrix **X**, and the columns of **X** be renormalized, so that they represent the conditional length distributions of the age-groups in the absence of any observations in the deleted length intervals. This adjustment would obviously be questionable in cases where it resulted in the removal from **X** of a substantial part but not all of an age-group's length distribution. Another disadvantage of the MMCS estimate not shared by the others is that there is no exact variance formula even for the constrained estimate.

To investigate the statistical behavior of the restricted estimates, a simulation study was performed with population parameters and sample sizes typical of marine commercial fisheries. Both OLS and MMCS estimates of age composition were computed from sample length distributions on the basis of a known length distribution matrix **X**, which for MMCS estimation was adjusted along with the length sample as described above whenever sample length frequencies of zero occurred.

Specifically, numerical trials were based on a von Bertalanffy growth curve with asymptotic length $\ell_\infty = 30$ cm, exponential coefficient $k = 0.5$/yr, and zero length at age $t_0 = 0$. Mean lengths at each age are given in Table 1. Within each age-group, the length distribution about

TABLE 1. Mean length ($\bar{\ell}_j$) at each age (j) in the artificial population studied. Successive differences are shown as multiples of the constant standard deviation of length at age $\sigma = 1.5$ cm.

Age j, yr	1	2	3	4	5	6	7
Mean length $\bar{\ell}_j$, cm	11.8	19.0	23.3	25.9	27.5	28.5	29.1
Difference $(\bar{\ell}_j - \bar{\ell}_{j-1})/\sigma$	—	4.8	2.9	1.7	1.2	0.7	0.4

the mean was normal with standard deviation $\sigma = 1.5$ cm, truncated at $\pm 3\sigma$, and the proportions falling in even 2-cm intervals were calculated accordingly to determine the elements of the length distribution matrix $\mathbf{X}$. For simplicity, knife-edge recruitment at age 1 was assumed, no animals older than age 7 occurred, and all sampling was done at integral ages. In each trial, a true age distribution $\boldsymbol{\pi}$ was generated randomly by drawing year-class strengths from a uniform [1, 10] distribution and applying an instantaneous total mortality rate $Z = 0.5$/yr as appropriate to each age-group. The corresponding true length distribution was then calculated as $\mathbf{p} = \mathbf{X}\,\boldsymbol{\pi}$, and a sample length distribution $\hat{\mathbf{p}}$ based on 1000 observations was obtained by repeatedly sampling a uniform [0, 1] distribution and assigning each observation to a length interval by inverting the true cumulative length distribution. Restricted OLS and MMCS estimates $\hat{\boldsymbol{\pi}}$ of the age distributions were computed and the errors $(\hat{\pi}_j - \pi_j)$ tabulated for each age-group (Table 2).

Bias in each estimate for each age-group is measured by a t-value, calculated as the mean error of estimate divided by its standard error. Some of these are large, but since the results reported in this paper include 42 such t-values, a few large ones are not significant in themselves. If both estimates were in fact unbiased and 42 independent t-values were computed (each based on a sample of 400), there would be a 5% chance of observing one or more greater in absolute value than about 3.26. Treating the estimates separately, there are 21 t-values for each and the 5% limit is about 3.12. Both limits are generous because to provide better comparisons only two sets of sample length distributions were involved in computing six sets of estimates, so the t-values are not really independent. The OLS estimate does not show any significant bias by the generous standard, but there is one t-value of -4.05 for the MMCS estimate, indicating significant bias. Despite this, the MMCS estimate has a smaller root mean squared error in almost every case, but the relative errors of the two estimates are close enough that one would not lose much precision by choosing the OLS estimate.

Both estimates perform well in distinguishing the relative abundances of the younger age-groups, whose length distributions overlap little with neighboring ones in the length distribution matrix. Similarly, both perform poorly among the older age-groups, the root mean squared errors approximating the mean values estimated. The last reasonably good estimate is for age 4 animals, whose mean length differs by 1.7 standard deviations from its nearest neighbor (Table 1). Other authors (Hasselblad 1966; McNew and Summerfelt 1978), whose work involved estimating the parameters of each age-group's length distribution as well as its relative abundance, have observed that relative age frequencies cannot be estimated with much precision among age-groups whose mean lengths differ by much less than two standard deviations. The results reported here show that this rule applies even when the component length distributions are known exactly.

None of the true age frequencies in these trials was zero, so all the zero elements among the estimates were false zeros, which as explained above will often occur in restricted estimates. As Table 2 shows, the OLS estimate produced somewhat more of them than the MMCS estimate: 471 compared with 427 among the same 2800 elements estimated. On the other hand, the MMCS estimate tended to concentrate its zeros; more than half occurred in one age-group, where significant bias was noted above.

Estimation with an Estimated Length Distribution Matrix

In practice the length distribution matrix $\mathbf{X}$ is never known exactly; it must be estimated from samples. To obtain unbiased estimates of the length distribution of an age-group, one must either measure a random sample from that age-group or measure and age all specimens in a random sample from the population as a whole. With rare exceptions, neither kind of data is ever collected.

For practical reasons, what is done instead is to determine the ages of a random sample from each length interval. This procedure, in conjunction with an independent sample estimate of the overall population length distribution, provides unbiased estimates $\{\hat{q}_{ij}\}$ of the proportion of animals that fall in length interval i and belong to age group j, for all age-length categories. The sum of these proportions for any age-group is therefore unbiased for the relative frequency of that age group:

$$E(\sum_i \hat{q}_{ij}) = \pi_j$$

This is the logic of the age-length key, and any systematic subsampling of a length distribution for age will provide the estimates $\{q_{ij}\}$.

Algebraically, an element x_{ij} of the length distribution matrix $\mathbf{X}$ can be written as

$$x_{ij} = q_{ij}/\pi_j = q_{ij}/\sum_i q_{ij}$$

so a natural estimate of x_{ij}, given the usual sampling data, is

$$\hat{x}_{ij} = \hat{q}_{ij}/\sum_i \hat{q}_{ij}.$$

In fact this is a biased estimate with an intractable distribu-

TABLE 2. Performance of restricted OLS and MMCS estimates in $n = 400$ trials with length samples of 1000 and **X** known.

Age j	1	2	3	4	5	6	7
Mean value of proportion estimated $\bar{\pi}_j$	0.3947	0.2404	0.1558	0.0937	0.0585	0.0344	0.0226
OLS estimate							
Mean error of estimate $\bar{e}_j = (\bar{\hat{\pi}}_j - \bar{\pi}_j)$	0.0007	0.0002	−0.0001	−0.0042	0.0076	−0.0041	−0.0002
Standard deviation of error s_j	0.0160	0.0154	0.0203	0.0374	0.0547	0.0418	0.0249
t-value for bias $t_j = \bar{e}_j\sqrt{n}/s_j$	0.90	0.31	−0.08	−2.25	2.80	−1.98	−0.16
Root mean squared error RMSE_j	0.0160	0.0154	0.0203	0.0376	0.0552	0.0420	0.0249
Relative error $\text{RMSE}_j/\bar{\pi}_j$	0.0405	0.0642	0.1305	0.4014	0.9433	1.2205	1.1022
False zeros in 400 trials	0	0	2	25	102	202	140
Root mean calculated variance	0.0159	0.0153	0.0208	0.0411	0.0762	0.1067	0.0536
MMCS estimate							
Mean error of estimate $\bar{e}_j = (\bar{\hat{\pi}}_j - \bar{\pi}_j)$	0.0001	−0.0002	0.0004	−0.0022	0.0069	−0.0079	0.0030
Standard deviation of error s_j	0.0156	0.0156	0.0197	0.0347	0.0480	0.0390	0.0240
t-value for bias $t_j = \bar{e}_j\sqrt{n}/s_j$	0.15	−0.31	0.39	−1.26	2.86	−4.05	2.47
Root mean squared error RMSE_j	0.0156	0.0156	0.0197	0.0348	0.0484	0.0398	0.0242
Relative error $\text{RMSE}_j/\bar{\pi}_j$	0.0396	0.0648	0.1265	0.3712	0.8279	1.1561	1.0719
False zeros in 400 trials	0	0	1	23	75	222	106

tion, but the bias must decrease as the sample sizes increase, and numerical results indicate that it will be negligible in commercial fishery assessments.

Numerical trials with the artificial population in this study consisted of drawing length samples of size M and age subsamples of size m, and then calculating $\{\hat{q}_{ij}\}$ and $\{\hat{x}_{ij}\}$ as above. Only when the sample sizes were reduced to $M = 100$ and $m = 10$ was any bias statistically detectable, and even then it was small. For practical purposes, therefore, it should usually be possible to estimate **X** without bias from standard age-length sampling data.

The procedure described above provides a nonparametric estimate of the matrix **X**, in that no assumptions are made about the form of the length distributions of the age-groups. In most other work on decomposing mixed length distributions, the component distributions have been assumed normal because the estimation problem included one or both of the parameters of each component length distribution as well as the relative age frequencies. In the problem considered here only the age distribution is to be estimated from the mixed length distribution, after the component distributions (i.e. the columns of **X**) have been estimated from other age-length sampling data. Either parametric or empirical estimates of these distributions could be obtained from the data, but the resulting estimates of age composition will be worse if the parametric approach is chosen and the assumption of normality is incorrect, as it sometimes is (McNew and Summerfelt 1978). Even if the component distributions are normal in nature, some of them are likely to be modified in commercial catches by gear selection or size limits. Empirical estimates do not involve either of the difficulties mentioned, and therefore will usually be preferred.

While for practical purposes the matrix **X** can be estimated without bias, it cannot be estimated without error. Random error in a regression matrix will normally produce systematic bias in the parameter estimates (Seber 1977). In this case it is at least certain that the estimates cannot all be biased in the same direction because the relative age frequencies are forced to sum to one, but the error in the regression matrix can be expected to increase the variance of the estimates.

The effect of estimating **X** was simulated in trials with the artificial population described above. As the first step in each trial, a random age composition was generated and its length distribution calculated from the true **X**. Next the true age distribution of each length interval was first calculated and then estimated from a random age sample (either 100 or 25) of that length interval. These sample proportions for each length interval were multiplied by the relative frequency of the interval in an independent length sample of 1000 to obtain estimated joint relative frequencies of age and length, namely the sample estimates $\{\hat{q}_{ij}\}$ referred to earlier, and finally an estimate $\hat{\mathbf{X}}$ of the length distribution matrix was calculated from them. The second step in each trial was to generate a new random age composition and its corresponding true length distribution, draw a random length sample of 1000 from that, and then estimate the age distribution on the basis of the estimate $\hat{\mathbf{X}}$ and the sample length distribution.

Tables 3 and 4 show the results obtained with age samples of 100 and 25 per length interval, respectively. As expected, these estimates give no more indication of bias than those computed with **X** known (Table 2); in fact they give less. Their variances are larger, but not by a great deal. In all three tables the estimates are reasonably good through age four and poor thereafter, and the MMCS estimate is slightly more precise than the OLS estimate.

These results suggest that pure sampling error in the estimate of the length distribution **X** will have little effect on the estimates of age composition, and that in most applications the major source of variance will be sampling error in the sample length distribution, with the large variances among the older age-groups resulting from the relatively close correspondence of their respective length distributions. In most applications, however, the true matrix **X** will be subject to some natural variability from year to year and some variability owing to the distribution of fishing and sampling within each year, both in addition to sampling error in any one year's estimate of **X**. So long as this variability in the true **X** is random, its effect will be the same in kind as the effect of random error in estimates of **X**, but the simulation results give no indication of its possible magnitude. The practical applica-

TABLE 3. Performance of restricted OLS and MMCS estimates in $n = 400$ trials with length samples of 1000 and $\mathbf{X}$ estimated from age subsamples of 100 per length interval.

Age j	1	2	3	4	5	6	7
Mean value of proportion estimated $\bar{\pi}_j$	0.3947	0.2404	0.1558	0.0937	0.0585	0.0344	0.0226
OLS estimate							
Mean error of estimate $\bar{e}_j = (\bar{\hat{\pi}}_j - \bar{\pi}_j)$	0.0001	0.0002	−0.0018	0.0003	0.0007	−0.0013	0.0019
Standard deviation of error s_j	0.0176	0.0179	0.0263	0.0458	0.0576	0.0415	0.0276
t-value for bias $t_j = \bar{e}_j\sqrt{n}/s_j$	0.09	0.21	−1.41	0.12	0.24	−0.60	1.36
Root mean squared error RMSE_j	0.0176	0.0179	0.0263	0.0458	0.0576	0.0415	0.0277
Relative error $\text{RMSE}_j/\bar{\pi}_j$	0.0446	0.0745	0.1690	0.4892	0.9839	1.2068	1.2259
False zeros in 400 trials	0	0	2	28	113	178	142
Root mean calculated variance	0.0158	0.0155	0.0209	0.0415	0.0622	0.0632	0.0408
MMCS estimate							
Mean error of estimate $\bar{e} = (\bar{\hat{\pi}}_j - \bar{\pi}_j)$	0.0001	0.0001	−0.0011	0.0015	0.0025	−0.0046	0.0024
Standard deviation of error s_j	0.0166	0.0173	0.0260	0.0449	0.0541	0.0380	0.0269
t-value for bias $t_j = \bar{e}_j\sqrt{n}/s_j$	−1.10	0.15	−0.82	0.69	0.92	−2.42	1.81
Root mean squared error RMSE_j	0.0166	0.0173	0.0260	0.0449	0.0541	0.0383	0.0270
Relative error $\text{RMSE}_j/\bar{\pi}_j$	0.0422	0.0718	0.1670	0.4791	0.9252	1.1126	1.1979
False zeros in 400 trials	0	0	0	28	98	175	123

tion presented below will show that variability in the true $\mathbf{X}$ may add substantially to the variance caused by sampling error alone, and it will also show that sampling error in the total length distribution may produce a substantially smaller variance when the component length distributions are irregular rather than smooth. For both reasons the results of simulation trials with the artificial population must be regarded as indicative rather than conclusive.

Variance Calculations

The restricted least-squares estimate will always be the constrained estimate from the full or some reduced model, in the latter case with zeros inserted for one or more age-groups. The variance−covariance matrix of the constrained OLS estimate from the chosen model can be calculated with the corresponding (usually reduced) regression matrix and the variance−covariance matrix of the sample length distribution (see Macdonald [1975] for the formula), but this procedure ignores the effect of carefully choosing one of many possible models in the first place, which as explained above is the essence of restricted estimation. Moreover, it makes no allowance for sampling error in the regression matrix, which will usually be present. For both reasons the calculated variance of the constrained estimate cannot be expected to measure accurately the actual variance of the restricted estimate.

Despite its shortcomings, this procedure is the best that can be done in the way of calculating variances. It provides no information whatever about the elements set to zero in a particular case, and it can only be applied in OLS estimation (and in weighted least-squares with fixed weights). It cannot be used in MMCS estimation because there the reciprocals of the observations are used as weights in calculating the estimates.

The last row of the OLS section in each of Tables 2, 3, and 4 shows the root mean calculated variance of each element of the OLS estimate in simulated trials. It refers only to those cases where a variance could be calculated (i.e. when that element of the restricted estimate was not zero). This figure can be compared with the empirical (simulated) standard deviation of each element, called the "standard deviation of error" in the tables, which refers to all trials.

Table 2 shows that when $\mathbf{X}$ is known, the calculated variance is, as it should be, a good estimate for the first four age-groups. The length distributions of all these are fairly well separated from their neighbors, so there cannot be much difference between the constrained and the restricted estimates in these elements. For the older age-groups, the root mean calculated variance is too high, exceeding the simulated standard deviation by more than 100% in two of the three groups. The reason for this is that the calculated variance measures the potential variability of the constrained estimate implied by the linear model and the observations, while some of this (namely the negative part of each element's theoretical distribution) is in fact suppressed by the process of restricting the estimate.

In Tables 3 and 4 the two errors in the calculated variance partly offset each other. The calculated value is somewhat too low among the first four age-groups because it takes no account of the random error in the regression matrix in these trials. This downward bias continues through the older age-groups but for the most part is not sufficient to counter completely the overestimation there.

On the basis of these results it seems safe to expect that even when there is some error in the estimated length distribution matrix, the calculated variance will be a reasonably accurate estimate for those age-groups whose mean lengths differ by, say, 1.5 or more standard deviations from their nearest neighbor. For the rest, the results presented here give no general guidance. Obviously if the length distribution matrix in a particular case had some columns closer than any in this study, the overestimation of variances could be larger than the maximum found here.

Insofar as the major source of variance in the restricted estimates is not sampling error in the regression matrix but rather in the observed length distribution, a good estimate can always be had when needed by simulation along the lines

TABLE 4. Performance of restricted OLS and MMCS estimates in $n = 400$ trials with length samples of 1000 and $\mathbf{X}$ estimated from age subsamples of 25 per length interval.

Age j	1	2	3	4	5	6	7
Mean value of proportion estimated $\bar{\pi}_j$	0.3993	0.2448	0.1487	0.0933	0.0586	0.0339	0.0214
OLS estimate							
Mean error of estimate $\bar{e}_j = (\bar{\hat{\pi}}_j - \bar{\pi}_j)$	−0.0021	−0.0009	0.0015	−0.0005	−0.0011	0.0016	0.0014
Standard deviation of error s_j	0.0163	0.0196	0.0335	0.0507	0.0577	0.0414	0.0284
t-value for bias $t_j = \bar{e}_j\sqrt{n}/s_j$	−2.61	−0.90	0.88	−0.19	−0.38	0.80	1.02
Root mean squared error RMSE_j	0.0164	0.0196	0.0335	0.0507	0.0577	0.0415	0.0284
Relative error $\mathrm{RMSE}_j/\bar{\pi}_j$	0.0411	0.0801	0.2254	0.5428	0.9848	1.2237	1.3300
False zeros in 400 trials	0	0	6	34	109	150	142
Root mean calculated variance	0.0158	0.0153	0.0204	0.0378	0.0522	0.0502	0.0300
MMCS estimate							
Mean error of estimate $\bar{e}_j = (\bar{\hat{\pi}}_j - \bar{\pi}_j)$	−0.0020	−0.0014	0.0024	−0.0004	0.0013	0.0004	−0.0002
Standard deviation of error s_j	0.0156	0.0194	0.0337	0.0501	0.0558	0.0398	0.0255
t-value for bias $t_j = \bar{e}_j\sqrt{n}/s_j$	−2.60	−1.43	1.43	−0.17	0.45	0.18	−0.13
Root mean squared error RMSE_j	0.0158	0.0194	0.0338	0.0501	0.0558	0.0398	0.0255
Relative error $\mathrm{RMSE}_j/\bar{\pi}_j$	0.0395	0.0794	0.2272	0.5371	0.9522	1.1755	1.1945
False zeros in 400 trials	0	0	5	31	100	143	138

described above. To do this one would treat the available estimate $\hat{\mathbf{X}}$ as true, fix an arbitrary age distribution $\boldsymbol{\pi}'$ (presumably one similar to the restricted estimate but with no zeros), calculate a hypothetical true length distribution $\mathbf{p}' = \hat{\mathbf{X}}\boldsymbol{\pi}'$, and then compute a series of restricted estimates of $\boldsymbol{\pi}'$ from random samples of the proper size drawn from $\mathbf{p}'$. The simplex algorithm is fast enough to provide empirical estimates in this way at reasonable cost.

Application to Grand Banks Cod

To provide a practical example and a check on the simulation results, the method was applied to data published by the International Commission for the Northwest Atlantic Fisheries (ICNAF) in its *Sampling Yearbook*. The exercise required a series of age−length sampling data on a single stock caught with the same gear, and to minimize the effects of different aging criteria and seasonal variations in length at age, the series chosen had to come from a single country and refer to catches taken in a single quarter of the year. Within these constraints the longest published series, among the more recent volumes consulted, referred to U.S. trawl catches of haddock on Georges Bank (ICNAF Division 5Z), but this series, in whole or in part, clearly showed an inverse relationship between year-class strength and growth rate. The basic assumption of the method was therefore violated and it simply could not be applied to this stock. The next best series referred to Portuguese trawl catches of cod on the Grand Bank (ICNAF Division 3L) in the second quarter, and covered the years 1967, 1970, and 1971 (ICNAF 1969, p. 86; 1972a, p. 69; 1972b, p. 62).

For each year, these data show the length composition of the catch for each month of the quarter and a single tabulation of all age readings by 3-cm length intervals. To standardize the age−length categories among years while maintaining reasonable sample sizes in all categories, all length composition and age−length data on fish less than 39 cm were ignored, and all data on fish greater than 78 cm were grouped. The truncation at 39 cm effectively eliminated the very small numbers of 3-yr-old fish in the data, and since fish aged 9 yr and older showed no clear differences in length distribution, they were all treated as a single group. Thus for each year the age−length sampling data were tabulated for a standard set of 14 length groups (39, 42, .., 75, 78+ cm) and six age−groups (4, 5, .., 8, 9+ yr). This table was converted to an age−length key, and the key was applied to the catch-weighted length composition of the entire quarter's sampled catch to estimate its joint age−length distribution and consequently its age distribution and underlying length distribution matrix. Finally the length distribution matrix for each of the three years was used to estimate the age distribution of the catch in each of the other two years from its length distribution by the restricted least-squares procedure. Only the OLS estimate was calculated, and its variance was estimated by simulation (as described above) rather than calculated. The results are shown in Table 5.

The age composition estimates in this practical example are unbiased (i.e. none of the mean errors is significantly different from zero), but beyond that they differ from the simulation results reported above in two ways:

i) The relative error of estimate is larger among the younger age-groups and smaller among the older ones than in simulation trials. The reason for this is simply the growth schedule of the stock. In the artificial population the mean lengths of the well-distinguished younger age-groups differed by more than two standard deviations, while among the oldest age-groups this difference was less than one standard deviation. In the cod data treated here, the apparent growth curve happened to be nearly linear and all the relative differences in the three length distribution matrices fell between 0.9 and 1.8 standard deviations, without even much tendency for the small differences to occur between older age-groups. Thus no age-group was either very well or very poorly distinguished by its length distribution, and the relative errors are about what would be expected from the simulation trials in such cases.

ii) For most age-groups the estimated variance is considerably less than the actual mean squared error. These variances

TABLE 5. Performance of the restricted OLS estimate when applied to data on cod in ICNAF Division 3L.

Age group j	4	5	6	7	8	9+
Year 1967						
True age distribution	0.067	0.303	0.327	0.143	0.069	0.091
Estimates with 1970 matrix	0.094	0.190	0.308	0.223	0.039	0.146
Estimates with 1971 matrix	0.092	0.092	0.432	0.250	0.066	0.068
Year 1970						
True age distribution	0.046	0.204	0.301	0.291	0.098	0.058
Estimates with 1967 matrix	0.019	0.308	0.327	0.254	0.071	0.020
Estimates with 1971 matrix	0.040	0.112	0.420	0.356	0.066	0.007
Year 1971						
True age distribution	0.084	0.382	0.341	0.147	0.036	0.010
Estimates with 1967 matrix	0.084	0.547	0.203	0.111	0.035	0.019
Estimates with 1970 matrix	0.142	0.320	0.356	0.087	0.058	0.038
Overall results						
Mean proportion estimated $\bar{\pi}_j$	0.066	0.296	0.323	0.194	0.068	0.053
Mean error of estimate	0.013	−0.034	0.018	0.020	−0.012	−0.004
Root mean squared error RMSE_i	0.030	0.134	0.087	0.069	0.024	0.038
Relative error $\text{RMSE}/\bar{\pi}_j$	0.46	0.45	0.27	0.35	0.35	0.70
Root mean simulated variance	0.016	0.033	0.050	0.054	0.032	0.010

were estimated in each case by simulating the effect of sampling error in the length composition, which in trials with the artificial population accounted for most of the variance in the estimates. In the example, however, the variance from this source is evidently smaller, while the variance due to differences among the estimated length distribution matrices is larger. Thus while the total mean squared error is about the same in the two sets of results, its components are different.

The larger variance due to the matrices simply means that the differences among them are greater than would be caused by sampling error alone — i.e. the true length distribution matrix is not the same for all three years. This is not surprising. There are a number of factors that can cause such differences, including the distribution of fishing and sampling over the time and area represented by each set of sampling data as well as the growth schedule of the stock. Some or all of these clearly varied in the present example, but the net effect on the estimates was not serious.

The smaller variance due to sampling error in the length compositions must result from the difference between the artificial population and the cod data in the form of the distributions of length at age. There is no other difference between the simulation trials with a known regression matrix reported above and the simulation trials that produced the variance estimates in this example. Evidently when the component length distributions are irregular empirical distributions rather than smooth normals, the computed total length distribution is more sensitive to the relative age frequencies, and therefore the estimates are less sensitive to variability in the sample length distribution.

Discussion

This paper has described how restricted least-squares estimates of age composition can be obtained in cases where gaps in age−length sampling data make it impossible to estimate age composition from length composition by the usual method of applying an age−length key. Restricted least-squares estimates are preferable to constrained least-squares estimates in that they guarantee nonnegative relative age frequencies, and they are preferable to restricted maximum-likelihood estimates in that they permit computation by a simplex algorithm rather than requiring the uncertain convergence of numerical search. In simulated trials with large length samples restricted OLS estimates were nearly as precise as MMCS estimates, and since the latter closely approximate maximum-likelihood estimates in these circumstances, it is unlikely that much precision will ever be lost in commercial fisheries assessments by choosing any form of least squares over maximum likelihood.

Of the three least-squares alternatives, MMCS estimates are the most difficult because they do not allow a straightforward variance calculation and require that the vector of observations and the regression matrix be adjusted whenever a zero occurs in the sample length distribution. In the author's view, these difficulties outweigh the slight gain in precision afforded by MMCS estimates over OLS estimates. A third alternative, weighted least-squares with fixed weights, would often provide estimates with nearly the same precision as MMCS estimates and none of the drawbacks.

While least-squares estimates will provide nearly the maximum precision attainable, this precision is not very great for age-groups whose mean lengths differ by much less than two standard deviations. In practice this will often mean that the relative abundance of older age-classes can only be stated precisely when their separate estimates are summed. This may seem to defeat the purpose of the exercise, but without going through the computations with all the distinct component length distributions specified, it would not be possible to determine the values of those elements that can be estimated

precisely. The very oldest age-classes will usually have to be treated as a single group from the outset in any case, since they will have nearly identical length distributions and therefore cannot be distinguished in length samples by any method. What all this means is that for the older age-groups in a population, even the best estimates to be had by finite mixture methods are a poor substitute for routine age–length sampling.

The method described here, unlike other finite mixture procedures that estimate some or all of the parameters of the component distributions as well as the mixing proportions, requires that the growth schedule not change, and that if exploitation is size selective, its level and pattern not change. These assumptions can obviously be relaxed without much affecting the estimates, since for the most part they concern the length distributions of the youngest age-groups which usually overlap little with their neighbors and therefore will be separated pretty accurately even if the true values differ somewhat from the estimates. In return for the assumptions, the method allows empirical rather than parametric estimates of the component length distributions, and it allows all available age–length sampling data to be combined to provide a more precise estimate of the length distribution matrix.

This paper has not attempted to deal quantitatively with the robustness of the method against violations of the basic assumption that the length distribution matrix **X** remains constant among years, primarily because of the number of factors that could cause some change and the number of possible interactions among them. The major hazards are the following:

i) Changes in growth schedule. Minor fluctuations will obviously have little effect, but a marked and systematic change in growth rate (e.g. a density-dependent change affecting either a single year-class or the whole stock) could produce a major change in the form of the matrix and therefore serious bias in the estimates.

ii) Seasonal effects. In all the simulation trials reported in this paper, both the age–length samples and the length samples referred to animals of integral age. In practice such data are collected piece by piece over the course of months while age-specific and overall length distirbutions are changing with recruitment and growth. All the distributions in the data and the model must therefore be regarded as averages over time. This averaging will reduce the precision of the estimates by increasing the standard deviations of the age-specific length distributions and it will cause some variability among years in the true value of **X**, which will depend not only on the growth schedule of the stock but also on the seasonal distribution of fishing and sampling. At the extreme, if sampling were concentrated at opposite ends of the major growth period in two years, the true values of **X** could differ by nearly a year's growth.

iii) Partial recruitment. In most fisheries, recruitment takes place over a fairly narrow size range, so that each year-class passes through a period when its larger members are exploited but its smaller members are not. This differential exploitation can be expected to leave its mark on the length distribution of the year-classs, and the shape of the mark will depend on the size at recruitment and the level of fishing mortality, so a change in either could change **X**. These marks would be important only where fishing took a significant part of a year-class during the period of partial recruitment, and they may fade with age.

iv) Segretation by size. If the animals tend to segregate by size, the true value of **X** will depend on the areas and depths fished each year. (An age–length key, properly applied, is unaffected by size segregation; this method is unaffected by age segregation.)

This method would be used only in cases where no age–length sampling data were available for some period, so an investigator could never know how the true value of **X** for that period compared with sample estimates of its value in other periods. In deciding whether or not to use the method, an investigator must look for significant differences among the estimates from other periods and draw on his knowledge of the stock and the fishery. The haddock data series referred to above is a case in which the method could not be used. The cod example shows that the method can produce reasonable estimates of relative age frequencies despite some variability in the length distribution matrix, but that their variances will be underestimated.

Acknowledgment

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Evidence of an Adaptive Basis for Geographic Variation in Body Morphology and Time of Downstream Migration of Juvenile Atlantic Salmon (*Salmo salar*)[1]

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RIDDELL, B. E. AND W. C. LEGGETT. 1981. Evidence of an adaptive basis for geographic variation in body morphology and time of downstream migration of juvenile Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 38: 308–320.

Differences in growth rate, proximate composition, body morphology, and time of downstream migration between two populations inhabiting tributaries of the Miramichi River, New Brunswick, that differed in distance from the head of tide, temperature, and flow velocity were studied. Rocky Brook, located 132.6 km above the head of tide, had lower temperatures and higher average flow velocities than Sabbies River, located 42.5 km above tide. Growth rate and proximate composition were similar between populations, but body morphology and time of downstream migration differed significantly between populations. Individuals from Rocky Brook had more fusiform bodies and larger paired fins than their counterparts in Sabbies River. Rocky Brook fish also left the tributary in the fall rather than in the spring as was the case in Sabbies River. The generality of the flow regime–body morphology relationship observed was tested and confirmed by predicting differences in morphology of juvenile salmon in other rivers based on a knowledge of their flow regimes. It is suggested that early migration by the Rocky Brook fish is related to the higher energetic costs of overwintering in that stream. We hypothesize that the phenotypic similarity in growth rate and proximate composition and geographic variation in body morphology and timing of migration have an adaptive basis.

Key words: geographic variation, polygenic traits, population biology, Atlantic salmon, morphology, migration, proximate composition, growth, adaptation

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Nous avons étudié les différences de taux de croissance, de composition par catégories de constituants, de morphologie du corps et d'époque de l'avalaison entre deux populations habitant des tributaires de la rivière Miramichi, Nouveau Brunswick, dont la distance depuis la tête des marées, la température et la vitesse d'écoulement diffèrent. Dans le ruisseau Rocky, situé à 132,6 km en amont de la zone des marées, les températures sont plus basses et les vitesses moyennes d'écoulement plus grandes que celles de la rivière Sabbies, située à 42,5 km en amont de la zone des marées. Le taux de croissance et la composition par constituants sont semblables chez ces deux populations, mais la morphologie du corps et l'époque de l'avalaison diffèrent de façon marquée. Les sujets du ruisseau Rocky ont un corps plus fusiforme et des nageoires paires plus grandes que ceux de la rivière Sabbies. En outre, les poissons du ruisseau Rocky quittent le tributaire à l'automne plutôt qu'au printemps, comme c'est le cas pour la rivière Sabbies. Nous avons vérifié et confirmé l'application générale de la relation observée entre l'écoulement et la morphologie du corps en prédisant des différences de morphologie chez des jeunes saumons habitant d'autres rivières, en se fondant sur l'écoulement de ces dernières. Nous croyons que la descente hâtive des saumons du ruisseau Rocky est reliée aux coûts énergétiques plus élevés d'un hivernage dans ce cours d'eau. Nous émettons l'hypothèse que la ressemblance phénotypique du taux de croissance et de la composition par catégories de constituants, d'une part, et de la variation géographique

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de la morphologie du corps et de l'époque de la descente, d'autre part, a un fondement d'adaptation.

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GEOGRAPHIC variation in species characteristics is a function of the genetic variability of individuals comprising local populations and of the environmental differences they experience in time and space. In fish populations, genetic sources of phenotypic variation have generally been deemed less important than environmental sources in determining interpopulation differences. The life history characteristics of the Salmonidae, however, suggest that genetic variation may contribute significantly to geographic variation in population traits. This hypothesis is well supported by Ricker's (1972) extensive review of hereditary and environmental factors influencing salmon populations and by the more recent demonstration of enzyme variation between salmonid populations inhabiting separate river systems (Allendorf and Utter 1979; Child et al. 1976; Payne et al. 1971; Møller 1970) and within a single lake (Ryman et al. 1979; Allendorf et al. 1976). The pronounced homing tendency of salmonids coupled with this accumulating evidence of spatial genetic variation between populations lends strong support to the hypothesis that salmon populations spawning in separate river systems are genetically isolated. Schaffer and Elson's (1975) demonstration that life history differences between North American Atlantic salmon populations have an adaptive basis further supports this view.

Genetic and environmental determinants of phenotypic variation within single river systems are less clearly defined. Until recently the decreased precision of salmonid homing to tributaries within a river system relative to river systems per se (reviewed in Stasko et al. 1973) was believed to impede the accumulation of genetic variation between demes. This view stems from Mayr's (1963) hypothesis that a period of ecological or spatial isolation is necessary to overcome the balancing effects of gene flow and natural selection before genetic variants can accumulate. Recent studies, however, suggest that gene flow and selection interact to set a lower limit on the scale of environmental changes required for differentiation (Spieth 1979; Felsenstein 1976). The assumption that straying is an index of gene flow between salmon populations may also be misleading. The relationship between gene flow and distance between populations is frequently leptokurtic and immigrants may have lower reproductive success than residents (see review in Endler 1977).

The potential for population-specific adaptation of juvenile Atlantic salmon to natal streams may be very high in light of the above suggestions and because presmolt mortalities are very high (Symons 1979). Support for this view is provided by studies of differences in the migratory behavior of juvenile Pacific salmonids which have a prolonged period of freshwater residence (Bowler 1975; Brannon 1972; Raleigh 1967, 1971).

In this paper we report the results of an investigation of the relative importance of genetic and environmental determinants of phenotypic variations in growth, body morphology, biochemical composition, and migratory behavior between populations of juvenile Atlantic salmon.

Materials and Methods

STUDY AREAS

The populations investigated inhabited two tributaries of the Main Southwest Miramichi River (Fig. 1), selected for study because of differences in their drainage basin morphology. We believed that these differences would result in differences in environmental conditions in the rearing habitats.

The sampling site on Sabbies River was located 42.5 km above the head of tide. Sabbies River has an estimated standing crop of 0.55 0^+ parr $\cdot$ m^{-2} (survey data, Freshwater and Anadromous Division, Department of Fisheries and Oceans), a vertical gradient of 5.05 m $\cdot$ km^{-1}, and a drainage area of 186.5 km^2 predominated by marshy terrain. Spawners are mainly 2-sea-winter salmon and older ($\simeq$75%) and enter the spawning tributary in late September or October. The sampling site in Rocky Brook was located 132.6 km above the head of tide. Rocky Brook has an estimated standing crop of 0.32 0^+ parr $\cdot$ m^{-2}, a vertical gradient of 11.5 m $\cdot$ km^{-1}, a drainage area of 94 km^2, and is fed by artesian lakes and springs. The spawning migration is a "spring-run" commencing in early June. Early arrivals are 2-sea-winter and older salmon; grilse (1-sea-winter) appeared in mid-June and eventually comprised 50% or more of the spawning adults.

ENVIRONMENTAL MONITORING AND DATA COLLECTION

To verify and quantify our assumptions concerning differences in rearing habitat, we monitored temperature and flow in both streams from June 13–December 4, 1975 and in Rocky Brook from May 28–October 30, 1976. Instrument malfunctions repeatedly restricted our attempts to duplicate the environmental monitoring in both streams during 1976. Temperature was monitored with Ryan continuous recording thermometers and flow velocity with General Oceanics (model 2010) current meters anchored in pools upstream from each sampling site. A Teledyne–Gurley current meter (model 665) was used to interpolate flow velocities at the sampling sites from flow velocities continuously recorded in pools. Mid-depth flow velocities were measured at $\sim$ 1-m intervals across three transects at the fish sampling sites on each sampling date. Regressions of measured sampling site flows against monitored flows were highly significant (Sabbies River $r^2 = 0.83$, $n = 45$; Rocky Brook $r^2 = 0.88$, $n = 63$).

The type and quantity of benthos in each stream were determined by collecting 5–0.11 m^2 Surber samples, coincident with each parr collection.

Growth rates and nine morphometric traits believed to be important in the hydrodynamics and station-maintaining ability of juvenile salmon were measured in each population. The morphometric measurements included head length (HL), head width (HW) and depth (HD), maximum body width (MBW) and depth (MBD), snout to the anterior insertion of the pec-

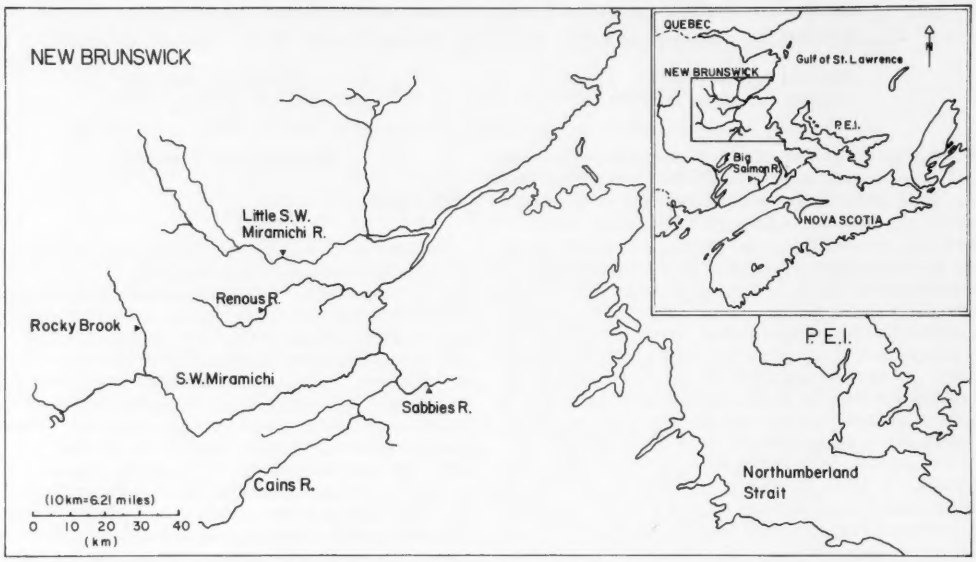

FIG. 1. Location of the New Brunswick rivers containing the Atlantic salmon populations in this study.

toral fins (SNAP) and pelvic fins (SNAPE), and pectoral and pelvic fin lengths (PL and PEL, respectively). All measurements were made to the nearest 0.1 mm following the criteria of Hubbs and Lagler (1967). Analysis of variance of differences between replicated measurements (15 individuals randomized and remeasured 5×) showed them to be nonsignificant (maximum deviation 3.2%).

We also determined weight, length, sex, stomach contents, and proximate composition of parr and downstream migrants from the two populations. All biochemical analyses were conducted on dried tissue. Whole fish were first homogenized and then dried to constant weight at 80°C. Moisture was determined by weight difference. The dried tissue was then ground to a fine powder and protein nitrogen was estimated from duplicated 0.08- to 0.1-g samples by micro-Kjeldahl digestion (Ogg 1960) followed by colorimetric Nesslerization reaction (Middleton 1960). Nitrogen values were converted to average protein value (N μg/g × 6.25). Total lipids were estimated from duplicated 0.25- to 1.0-g (dry weight) samples by 6 h refluxing in a Soxhlet apparatus with 100% chloroform and measured gravimetrically. Ash was determined by combusting up to 1.0 g of dried tissue overnight at 550°C.

Lipid, protein, and total sugar content of the benthos were also assayed. Each Surber sample was sorted into groupings of Ephemeroptera, Plecoptera, Trichoptera, and others (largely Dipterans), and dry weight biomass of each group was determined. All dried samples for each sampling period within each stream were pooled for biochemical analysis. Protein was analyzed by the technique utilized for fish tissue. Lipid was extracted by a modified Bligh and Dyer procedure and measured gravimetrically (Mayzaud and Martin 1975). Soluble sugars and glycogen were extracted using the method of Van Handel (1965), then pooled for colorimetric analysis of total sugars (Dubois et al. 1956) with a D-glucose standard.

The study began with samples of the 1^+ age-class in each population. Samples were obtained at 8-wk intervals beginning in June 1975 and continuing until the downstream migration of most of the age-class occurred (Sabbies River, May 1976; Rocky Brook, October 1976). Parr were electroseined and downstream migrants were trapped in a modified box net (Hare 1973). All fish were frozen on dry ice immediately after capture and kept at −40°C until analysis.

Morphometric data was also collected from 1^+ parr inhabiting three other streams of known seasonal flow profiles. Seven to 10 yr of daily mean flow velocities for the Little Southwest Miramichi (LSWMR), Renous (RR), and Big Salmon Rivers (BSR) were compared (Water Survey of Canada data, Fig. 1, 2). Relative body morphologies predicted from the flow data were used as a test of hypotheses developed during this investigation. Fifty parr were collected from LSWMR and RR and two 50-parr samples were collected from BSR, one from the headwaters (BSU) where flows are moderate and a second near the outlet (BSL) where flows were greater. The flow pattern for BSR in Fig. 2 would be more representative for the BSL sample. A physical description of BSR is included in Jessop (1975).

STATISTICAL METHODS

Discriminant analysis was used to define overall morphological variation between populations. Rao's (1952) F ratio test was used to test the significance of the discrimination achieved. In all statistical analyses concerning morphometric data, except the comparisons of downstream migrants, body

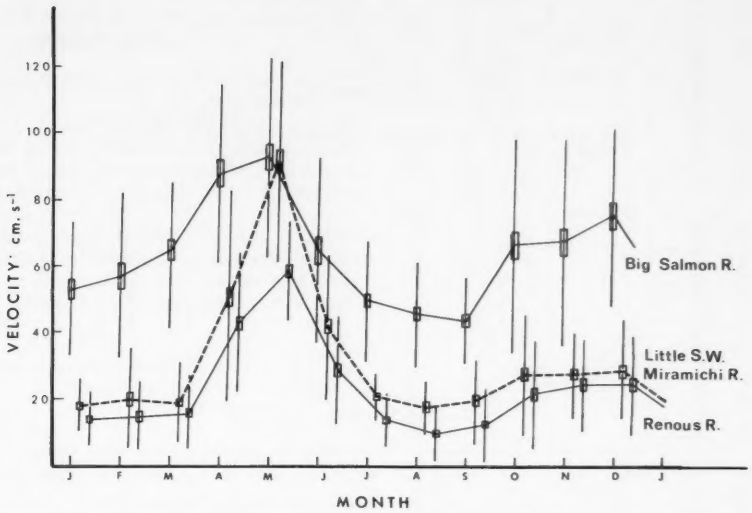

FIG. 2. The mean monthly flow velocity (± 95% CI about the mean and 1 SD) for 12-yr data on the Little Southwest Miramichi River, 10-yr on the Renous River and 7-yr on the Big Salmon River.

length was standardized to the grand sample mean length. This procedure minimizes variation induced by allometric growth and differences in mean size of individuals between samples (Gould 1966; Sprent 1972). The standardization technique, outlined in Thorpe (1976), involved log−log regressions of morphometric traits against fork length within each population. The antilog of standardized data was used in all analyses. The first axis of many multivariate techniques applied to population data is commonly a size function (Gould and Johnston 1972). Standardized data therefore permitted a clearer interpretation of the morphological differences between populations. Further, Blackith and Reyment (1971) and Van Valen (1974) have drawn attention to possible errors in some multivariate statistics when using highly correlated variables. Length standardizations accounted for 50−55% of the total variance in our data and substantially reduced the level of correlations between morphometric traits. Standardized discriminant functions were calculated using the SPSS version 7.2 Discrimination program (Nei et al. 1975). This program involves stepwise variable entry for which we employed the Wilks lambda criteria. The equality of within-group variance−covariance matrices was tested by the SAS−DISCRIM program (Barr et al. 1976).

The influence of environmental variation on growth rate, weight−length relationships, and proximate composition was investigated by stepwise multiple regression analyses, using the SPSS−Regression program (Nei et al. 1975). A dummy variable ($P_1 = 1$) was created to define fish from Sabbies River, and five environmental variables were utilized: the 5-d mean temperature (°C) and flow velocity ($m \cdot s^{-1}$) previous to the sampling dates, the benthic biomass (diet, g dry wt $\cdot m^{-2}$), and corresponding lipid (DL = g dietary lipids) and protein weights (DP = g dietary protein) from benthos samples collected on the sampling day.

Results

Environmental Variation

Mean temperatures were consistently lower and mean flow velocity consistently higher in Rocky Brook than in Sabbies River (Fig. 3). Temperature was inversely related to flow velocity ($r = -0.33$, $n = 81$, $P < 0.01$). Annual variation in flow patterns can be significant as observed in Fig. 3 between the 1975 and 1976 records for Rocky Brook.

Whether between-river variation in flow velocities exceeds temporal variations within rivers will depend upon the degree of differences in the drainage morphologies of the streams compared. Predictability, as defined by Colwell (1974), of within-river temporal variations in flow was evaluated from the longer-term flow records available for the BSR, RR, and LSWMR. The predictability of flow velocity decreases and the seasonal patterns of flow become increasingly pronounced as the stream gradient and catchment area decrease (Riddell 1979).

Differences in flow observed in 1975 are probably representative of long-term environmental differences in light of the twofold greater stream gradient in Rocky Brook. The degree of between-stream difference relative to within-stream variation is, unfortunately, not definable. Temperature differences are believed to reflect the nature of each stream's headwater source, as well as flow rates.

Food Items and Benthos Samples

The diets of Rocky Brook and Sabbies River parr were qualitatively different but increased in similarity between June 1975 and February 1976 (Table 1). Seasonal changes in the predominant dietary items were similar in the two streams

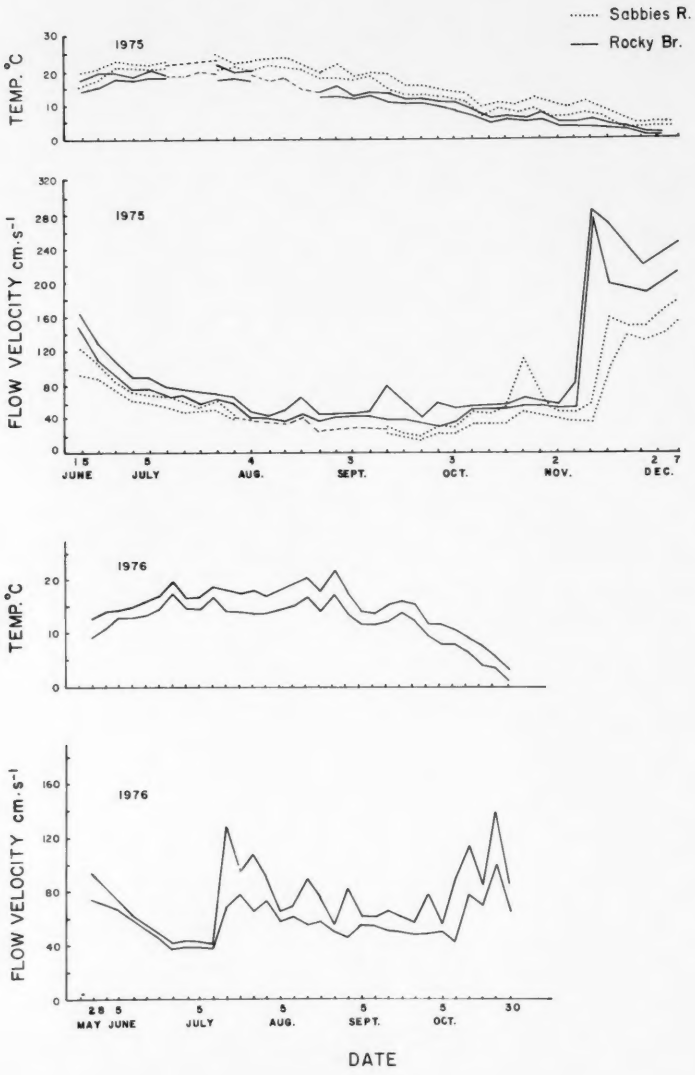

FIG. 3. Ninety percent confidence limits about 5-d geometric mean temperature and flow velocity within Sabbies River and Rocky Brook for 1975 and 1976. Broken lines represent mean values during periods of instrument failure; estimation procedures are described in text.

but were influenced by the environmental differences observed. The lower temperatures and higher flow velocities in Rocky Brook retarded emergence of *Simulium* sp. and appeared to effect changes in the trichopteran fauna.

Dry weight biomass of benthic invertebrates, an index of food abundance, did not differ consistently between streams. Pooled estimates of dry weight biomass indicated that Sabbies River benthic biomass was more variable seasonally but no significant difference (t-test) in mean annual biomass was observed (g dry wt · m^{-2} Rocky Brook = 1.1 ± 0.3;

Sabbies River = 1.7 ± 1.0, $P = 0.17$). The proportions of lipid, protein, and total sugar in the insect biomass were similar in the two streams (Table 1).

Growth Rate and Weight–Length Relationships

Sample mean lengths of parr and downstream migrants were consistently greater in Sabbies River (Fig. 4). Daily growth increments during June through October 1975 (Fig. 4) and weight–length relationships for parr were very similar in the two populations.

The regression best describing growth rate (eq. 1) showed that the regression slopes of length (LT) against age (d) within populations did not vary significantly but that intercept values are significantly different between populations (partial correlation $P_1 = 0.48$).

$$(1)\quad \text{Log}_{10}\,\text{LT cm} = 0.00034\,\text{d} - 0.095\,\text{flow} + 0.0504\,P_1 - 0.003\,\text{T°C} + 0.045\,\text{diet} + 0.7982 \qquad R^2 = 0.82$$

Inclusion of the environmental parameters plus the dummy variable P_1 in the multiple regressions increased R^2 by 0.2837. Flow velocity accounted for 66% of the explained residual variance and had a negative partial correlation with length ($r = -0.64$). The smaller daily growth increments for Rocky Brook parr suggests that flow velocity was the primary environmental source of between-population differences in growth rate during 1975. The greater intercept value in Sabbies River, however, indicates that growth during the 0^+ year is largely responsible for the greater absolute size of parr collected from Sabbies River.

In contrast to growth rate, multiple regressions of weight (WT) versus length and the environmental variables (eq. 2,3) showed the weight–length relationship to be less responsive to environmental variation. Temperature was the only parameter that increased R^2 significantly.

$$(2)\quad \text{Log}_{10}\,\text{WT} = 2.878\,\text{log}_{10}\,\text{LT} - 1.8425 \qquad R^2 = 0.91$$

$$(3)\quad \text{Log}_{10}\,\text{WT} = 2.9904\,\text{log}_{10}\,\text{LT} + 0.0044\,\text{T°C} - 2.0034 \qquad R^2 = 0.92$$

Growth rates of 1975 1^+ parr and the resulting 1976 2^+ age-class in Rocky Brook were also similar (t-test for equality of slopes, $P = 0.96$). Daily growth increments did, however, show a consistent decrease with age.

Timing of Downstream Migration

Juvenile salmon from Sabbies River and Rocky Brook differed greatly in the timing of downstream migration. Large parr left Rocky Brook in late October. Sabbies River migrants moved downstream in May and at that time exhibited the characteristic body form, coloration, and biochemical composition of smolts. Trends in the environmental stimuli believed to be important regulators of migratory activity in juvenile salmon (temperature, flow velocity, and photoperiod; reviewed by Hoar 1965, 1976) were very different in the two tributaries at the time of emigration. Flow

TABLE 1. Diet composition and benthic invertebrate biomass, composition, and biochemical components compared between streams and seasons. Table entries for Ephemeroptera, Plecoptera, Trichoptera, and others are % frequency of occurrence in diet/% dry weight of benthic sample composition.

	June		July		October		December		February	
	Rocky Brook	Sabbies River	RB	SR	RB	SR	RB	SR	RB	SR
Ephemeroptera	91/38	79/45	96/17	64/15	94/66	32/5	62/60	72/22	41/22	37/5
Plecoptera	30/38	79/16	1/29	6/8	32/3	42/32	50/6	61/71	38/9	70/55
Trichoptera	25/21	67/31	22/39	92/52	45/24	62/57	33/32	50/10	14/58	19/28
Other (Diptera)	87/3	77/7	91/15	35/25	21/6	19/6	22/3	10/8	3/10	7/12
Mean ± 1 SD benthic biomass g dry wt · m^{-2} ($n = 5$)	1.43±0.61	1.22±1.07	1.01±0.58	1.26±0.66	1.14±0.63	2.47±0.97	0.71±0.40	0.61±0.73	1.16±0.59	3.08±4.58
% lipid ± 1 SD ($n = 3$)	16.4±0.58	18.9±0.42	15.4±0.44	13.2±0.31	15.5±0.26	13.22±1.3	12.8±0.59	14.8±0.43	17.6±1.9	22.2±2.3
% protein ± 1 SD ($n = 3$)	45.6±0.66	47.5±1.10	42.8±1.1	38.6±0.74	49.0±2.8	47.1±2.0	50.1±0.61	57.9±1.78	49.3±1.3	50.1±1.77
% total sugars ± 1 SD ($n = 3$)	2.14±0.011	2.84±0.04	3.24±0.012	2.3±0.014	2.0±0.02	1.83±0.011	5.25±0.03	5.5±0.04	3.64±0.016	5.7±0.021

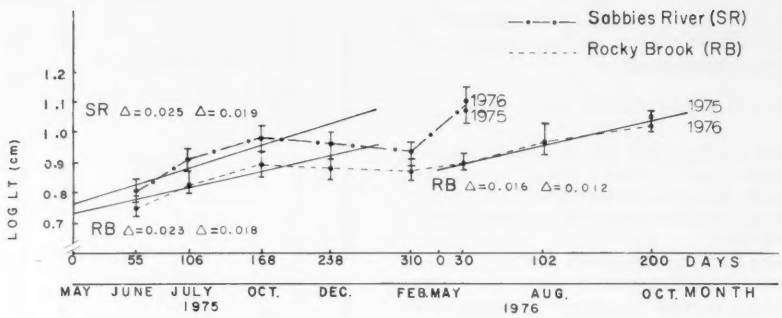

FIG. 4. Mean fork length (±1 SD) for parr collected during 1975–1976. Length (cm) against age (days) regressions are superimposed on sample means; May 1 was subjectively set at day zero. Delta = daily growth increment between sampling dates (cm · d^{-1}).

velocities declined during the spring smolt migration from Sabbies River (averaged flow velocities decreasing from >1 m · s^{-1} to 0.6 m · s^{-1}) but increased during the movement of large parr from Rocky Brook (0.4 m · s^{-1} up to 0.7 m · s^{-1}). Mean daily temperatures during the smolt migration ranged from 8 to 12°C but were 2–7°C during the autumn movement of large parr (Riddell 1979).

Downstream migrant fish were sampled in both tributaries in May–June and October–November 1975. During October 1976 a duplicate sample of Rocky Brook large parr was collected but sampling efforts were concentrated in Sabbies River. No fall movement of large parr was observed in Sabbies River and migration periods were consistent within streams between years. The absence of migrants during the spring in Rocky Brook and fall in Sabbies River is not believed to be due to ineffective trapping since large numbers of other species were caught (trout, cyprinids, catastomids, and eels).

Rocky Brook migrants were significantly smaller than smolts from Sabbies River (t-test, $P < 0.01$) but were older (Table 2; mean age of smolts = 2.37 yr, mean age of large parr = 2.46 yr). Although 42% of the male migrants in Rocky Brook were precocious, the sex ratio was 1:1. The incidence of precosity increased from 37% in the first third of the run to 58% in the final third. A pronounced decline in testes weight also occurred (5–10% to 0.4–3.0% of wet body weight). No precocious male smolts were detected in Sabbies River but the predominance of females may indicate overwinter mortalities or different migratory patterns in precocious male parr (Saunders 1972). Estimates of precocious development in Sabbies River parr during 1975 varied from 12 to 33%.

Proximate Composition of Parr

No significant differences occurred in the moisture, protein, and lipid content of parr from the two populations (Fig. 5). Body size relationships accounted for greater than 94% of the total variance in water, protein, and ash weights but only 62.5% of the variance in lipid weight (Table 3A). The proportion of the explained variance in lipid weight was significantly increased by including moisture as an independent variable ($R^2 = 0.87$, eq. 12). The partial correlation of lipid weight with moisture after controlling for body weight was $r = -0.78$ (F ratio test, $P < 0.0001$).

$$(12)\quad \text{Log}_{10}\,(\text{lipid g}) \pm 0.0114 = 1.348\ \text{Log}_{10}\,\text{WT} - 1.829\ \log_{10}\,\text{LT} - 9.692\ \text{moisture} + 7.374 \qquad n = 436 \quad R^2 = 0.87$$

The residual variation in protein, ash, and lipid, after controlling for body size, was partially accounted for by variation in temperature and flow velocity. Temperature accounted for 81% of the explained residual variation in lipid content; however, the seasonal intrapopulation variability in lipid content was greater than variation in lipid content between populations (Fig. 5). Flow velocity accounted for more than 50% of the unexplained variance in water and protein weights. The proportion of the total phenotypic variation in protein and water content attributed to environmental parameters was very small in comparison to the influence of body size.

Proximate Composition of Downstream Migrants

Protein and moisture weights were greater and ash and lipid weights less in the smolts from Sabbies River when compared with resident parr from both rivers or with the fall migrants from Rocky Brook (Fig. 5). Regressions of body size with biochemical components in the migrants were calculated independent of the parr samples because of the physiological and growth changes that occur during smoltification (Hoar 1976). Protein weight was significantly higher ($P_1 = 0.0235$, F ratio test, $P < 0.001$) in the smolts than in the migrant large parr from Rocky Brook, suggesting that food consumed during the spring was assimilated as protein. The reduction in lipid levels over the same interval may have been due to the increased growth rate in the spring (Hoar 1976), or as Hoar has noted, changes in lipid may also be an essential aspect of smoltification. These metabolic changes during smoltification would account for the signs of the P_1 coefficient in the regressions given in Table 3B. The proximate composition of Sabbies River spring smolts was very similar to the values reported for Baltic salmon smolts by Malikova (1957).

TABLE 2. Sample mean length and weight (±1 SD), sex ratio, and age composition of downstream migrant large parr from Rocky Brook and smolts from Sabbies River. MP = precocious male.

Migratory period	*n*	Mean length ± 1 SD cm	Mean weight ± 1 SD g	F:M:MP	$1^+:2^+:3^+$
Sabbies River May 1975	88	12.58±0.84	20.39±4.02	0.72:0.28:0	0:0.73:0.25
Sabbies River May 1976	55	13.00±1.11	23.37±6.11	0.52:0.47:0	0:0.36:0.64
Rocky Brook October 1975	100	11.43±0.83	15.54±3.66	0.54:0.25:0.21	0:0.76:0.24
Rocky Brook October 1976	55	11.09±1.09	14.72±4.88	0.40:0.36:0.24	0.18:0.69:0.13

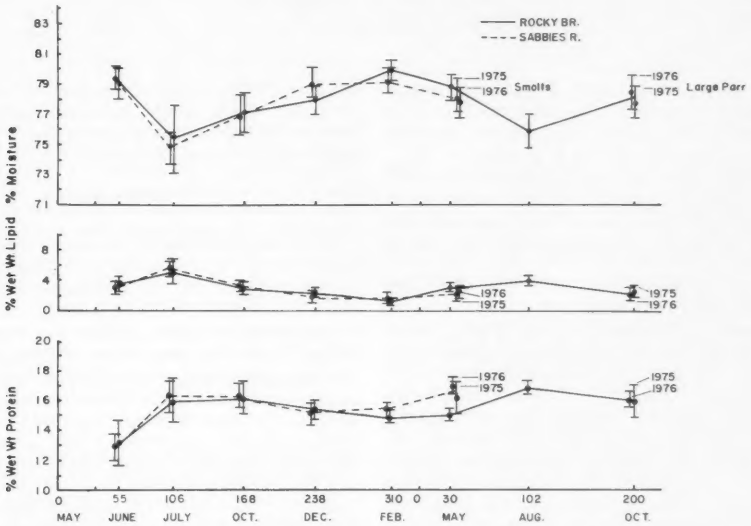

FIG. 5. Mean moisture, lipid, and protein (±1 SD, % wet weight) of juvenile Atlantic salmon from Rocky Brook and Sabbies Rivers in response to growth and seasonal environmental variation.

The proximate composition of fall migrant parr in Rocky Brook was similar to the resident parr within the stream. Lipid weight was greater in the migrants ($\Delta = +1.5\%$, *t*-test, $P < 0.01$) than would have been predicted from equation 12, but no significant differences occurred between levels of protein and ash. Rocky Brook fall migrants did not exhibit the lipid–protein relationships typical of smolts. Evropeizeva (1959) has argued, on the basis of proximate composition and histological studies, that precocious development and smoltification are biologically incompatible and cannot occur simultaneously. This being the case, the high frequency of precocious males in Rocky Brook, and the fact that the sex ratio was 1:1 during emigration (Table 2), suggests that the fall migrants were not undergoing smoltification at the time of emigration.

BODY MORPHOLOGY

Consistent significant differences in body morphology occurred between populations (Fig. 6). A normal distribution ($N[d/2,1]$) about the population centroids in each standardized function was assumed (Fig. 6) to facilitate the demonstration of the overlaps in morphological variation. The morphological traits which vary between populations and a measure of their relative importance in the discrimination can be derived by comparing the sign and weight of coefficients in a standardized function with the univariate means of traits within each population. For example, the larger coefficients for pectoral fin (PL), pelvic fin (PEL), and head length (HL) in the equations of Fig. 6 are contrasted with the coefficients for body depth or weight. Rocky Brook parr had significantly larger head and fin lengths (minimum *t* value = 5.36 for PEL, $P < 0.01$) and their maximum body depth was significantly smaller, (*t*-test, $P < 0.01$) (Riddell 1979). The downstream migrant comparison (Fig. 6C) was not standardized for body size variation. Body weight was consequently an important variable in equation 15 but the large positive coefficient for PL again indicates Rocky Brook large parr have longer pec-

TABLE 3. Multiple regressions of body size vs. water content, protein, lipid, and ash weight (g wet wt) for parr (A) and downstream migrants (B). A dummy variable (P_1 = 1 = Sabbies River) and moisture (M = water wt/wet body wt) are included as independent variables in B.

A. $\text{Log}_{10}(Y) \pm (t_{0.05} \cdot Sy/\sqrt{n}) = b_1 \log_{10}WT + b_2 \log_{10}LT + \log_{10}a$			
Water (g) $\pm$ 0.001 = 0.988 $\log_{10}$WT − 0.10	n = 486	R^2 = 0.99	(4)
Protein (g) $\pm$ 0.004 = 1.074 $\log_{10}$WT − 0.874	n = 447	R^2 = 0.97	(5)
Lipid (g) $\pm$ 0.019 = 1.964 $\log_{10}$WT − 3.126 $\log_{10}$LT + 0.563	n = 436	R^2 = 0.62	(6)
Ash (g) $\pm$ 0.009 = 1.037 $\log_{10}$WT − 1.523	n = 103	R^2 = 0.94	(7)
B. $\text{Log}_{10}(Y) \pm (t_{0.05} \cdot Sy/\sqrt{n}) = b_1 \log_{10}WT + b_2 \log_{10}LT + b_3 P_1 + b_4 M + a$			
Water (g) $\pm$ 0.0006 = 0.975 $\log_{10}$WT + 0.004 P_1 − 0.079	n = 296	R^2 = 0.99	(8)
Protein (g) $\pm$ 0.0023 = 0.917 $\log_{10}$WT + 0.228 $\log_{10}$LT + 0.024 P_1 − 0.838 M − 0.288	n = 296	R^2 = 0.98	(9)
Lipid (g) $\pm$ 0.0124 = 1.636 $\log_{10}$WT − 2.175 $\log_{10}$LT − 0.03 P_1 − 11.757 M + 9.057	n = 253	R^2 = 0.82	(10)
Ash (g) $\pm$ 0.0058 = 0.751 $\log_{10}$WT + 0.532 $\log_{10}$LT − 0.018 P_1 − 1.772	n = 154	R^2 = 0.93	(11)

toral fins than Sabbies River smolts. Indeed, even without standardization the mean pectoral fin length ± 1 SE (2.24 $\pm$ 0.15 cm, n = 150) of Rocky Brook large parr was larger than that of Sabbies River smolts (2.21 $\pm$ 0.15 cm, n = 143).

Generalizing our results, we found that the juvenile salmon of Rocky Brook were less robust in body form and had greater head length during the parr phase. They consistently had longer pectoral and pelvic fins than did the parr from Sabbies River.

For the 1^+ parr collected during 1975, 37% of the total phenotypic variation in body morphology was attributed to differences between populations. Evidence of spatial variation in body morphology within a stream is given by the reduced distance between group centroids (1.209−1.022; Fig. 6 A,B) when the 2^+ parr of the 1974 year-class in Rocky Brook were pooled with the 1^+ 1975 samples. This reduction in the distance between sample means may have resulted from mixing, during the winter, of fish reared in different habitats within Rocky Brook. The significant difference in body morphology between populations was maintained (F ratio test, $P < 0.01$) in spite of this effect. A minimum estimate of the total phenotypic variation in body morphology attributed to population differences is 20.8% (Fig. 6B). Annual variation in the degree of divergence in body morphology between populations was small (Fig. 6C). The shift in distributions between years suggests year-to-year environmental differences may act to influence the overall expression of morphometric traits. This effect may be moderated through its influence on growth (Martin 1949).

The apparent relationship of a more streamlined body form and longer paired fins with greater flow velocity was tested by predicting the relative body forms of juvenile salmon in BSR, RR, and LSWMR. Our a priori prediction was that BSR fish would be more streamlined and/or have larger fins than those of the RR or LSWMR (see Fig. 2). A similar relationship should exist between LSWMR and RR fish. The smaller head and more streamlined body form of both BSR samples relative to the Miramichi populations, and longer paired fins of the BSL sample with respect to the BSU fish agreed with our predictions.

Head width predominated the first axis of Fig. 7, and BSL and BSU parr had significantly narrower heads than parr from LSWMR and RR ($\bar{x}$ (LSWMR + RR) = 0.92 $\pm$ 0.04 cm; $\bar{x}$ (BSL + BSU) = 0.86 $\pm$ 0.04, t-test, n = 100, $P < 0.01$). Parr from LSWMR and BSU had more robust bodies than parr in RR and BSL, respectively (axis 2, Fig. 7). Fin sizes were equal in LSWMR and RR samples.

Discussion

In this study we related the phenotypes of several polygenic traits in juvenile Atlantic salmon to environmental differences in rearing habitats. Mean daily temperatures in Rocky Brook were, on average, 0.66× those in Sabbies River and showed greater variability (1.6× coefficient of variation (CV) in Sabbies River). The source waters of Rocky Brook are artesian lakes and springs, whereas Sabbies River drains a large marshy area of eastern New Brunswick. Rocky Brook, therefore, may be more dependent upon daily solar heating to maintain its daily temperature. Flow velocities in Rocky Brook were consistently greater than in Sabbies River, being ~ 1.25× greater on a daily basis and also having greater variability (1.27 × CV). The headwater marshes of Sabbies River and the more extensive channelization of Rocky Brook would both contribute to the greater flow variation in Rocky Brook (Leopold et al. 1964).

Many other aspects of stream habitat, including food and space, the proportion of substrate types, pool:riffle ratios, and the availability of cover, are known to be important to salmonid biology (see review in Northcote 1969; Mundie 1974). We believe, however, that the relatively small differences in underyearling parr densities and benthic biomass between Rocky Brook and Sabbies River are of limited importance to our analysis since growth increment, weight−length relationships, and proximate composition were very similar between these populations

The phenotype exhibited by an organism represents the expression of a complex of covarying traits developed under a specific sequence of gene−environment interactions. In natural populations, the phenotypic variance of each trait in the complex is due to environmental and genetic sources. The relative importance of these sources varies with the tolerance of individual genotypes to environmental variation (Dobzhansky 1968) and with the selective pressure exerted on the trait (Fisher 1930). Their relative importance has also been hypothesized to vary with the scale of spatial and temporal

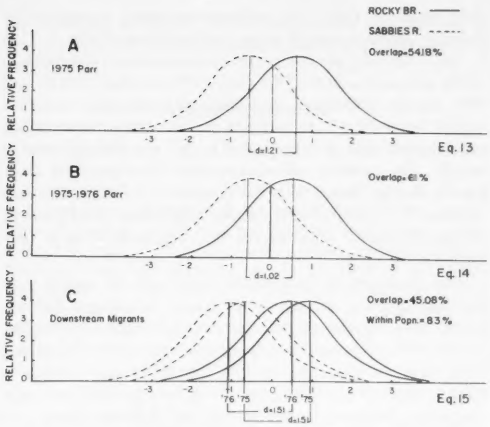

FIG. 6. Discriminant function analyses describing the morphometric variations between Rocky Brook (—) and Sabbies River (---). Equations (13–15) are standardized discriminant functions, d = distance between population mean values in units of pooled within-group standard deviations. The canonical correlation (R^2) indicates the proportion of the total variance attributable to differences between populations.

Eq. 13; $\hat{X}$ = 0.25HD − 0.22MBW − 0.67MBD + 0.21HL + 0.37PL − 0.13SNAPE + 0.29PEL R^2 = 0.37

Eq. 14; $\hat{X}$ = 0.24HD − 0.11HW + 0.4MBW − 0.87MBD + 0.53HL + 0.3PL − 0.14SNAPE + 0.43PEL R^2 = 0.21

Eq. 15; $\hat{X}$ = 0.15HD − 0.14HW − 0.15MBW + 0.26MBD + 0.66PL − 0.31SNAPE + 0.2PEL − 0.89WT R^2 = 0.64

variability in the environment (Levins 1965, 1968). In studies of geographic variation, the environment is often viewed as a spatial gradient superimposed with temporal variability. The genetic control of individual traits within a population may then be balanced between selection resulting from spatial variation and genetic variation that is maintained by the temporal uncertainty of the environment. In studies such as ours where a limited number of populations are studied, it is necessary to designate individual environments as discrete spatial units and assume that the environmental variation observed between them is representative of their long-term spatial differences. If the long-term spatial differences exceed the within-population temporal variation and are selective, Bryant (1976) suggests that selection would be expected to result in adaptations to these long-term conditions.

The stability of the 1^+ growth rate and proximate composition of juvenile salmon from Rocky Brook and Sabbies River indicates that the environmental differences observed between streams had a very limited effect on the phenotypic expression of these traits. Seasonal variation in temperature and flow did, however, exert a pronounced effect on all growth and biochemical traits (Fig. 4, 5).

The limited differences in growth rate between populations which were observed were largely attributed to variation in flow velocity (eq. 1). The effect of flow on growth in natural environments has been difficult to assess presumably because

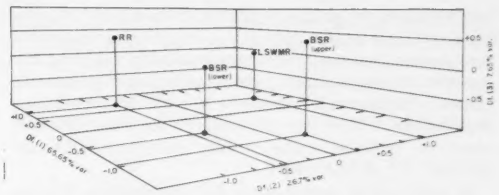

FIG. 7. Discriminant analysis comparing body morphology between the four test samples collected from the Renous River, Little S.W. Miramichi, Upper and Lower Big Salmon River. Eighty-four percent of the total variance is accounted for by the three functions, the % variance along each axis is the % of the explained variance each axis describes.

Df(1) = 0.06WT − 0.09HD − *0.79HW* + 0.145MBW + 0.179MBD − 0.27HL − 0.18SNAP − 0.2PL − 0.04SNAPE + 0.3PEL; R^2 = 0.656

Df(2) = *1.15WT* − *0.32HD* + 0.28HW − *0.93MBW* + 0.10MBD − 0.4HL + 0.39SNAP + 0.11PL − 0.2SNAPE − 0.29PEL; R^2 = 0.434

Df(3) = *0.33HD* + *0.43HW* − 0.14WT + OMBW − 0.06MBD − 0.32HL + 0.02SNAP − 0.18PL − *0.60SNAPE* − *0.41PEL*; R^2 = 0.183

the average flow velocities measured in streams do not necessarily reflect the velocities actually experienced by the fish. In the genus *Salmo*, riffle-inhabiting juveniles maintain a close proximity to the substrate (see Fig. 2B, Symons 1976) and dart into the higher flow velocities to feed (Kallenberg 1958). Since current velocity in streams is proportional to the logarithm of the depth (Hynes 1970) and effective refuges from the current are created by eddies behind rocks (Jaag and Ambühl 1964), such behavior in parr can significantly alter the flow regime experienced. The principal effect of flow on growth may operate through its effect on the energetic cost of the feeding bursts and/or on the efficiency of prey capture at different flow velocities. During our observations of parr holding feeding positions in a riffle, we frequently saw them being displaced downstream and returning to their holding position. Differences in flow velocity experienced during such events could alter their rate of net energy gain thereby influencing growth. Symons and Heland (1978) recently suggested that average annual flow velocity provides a useful index of the quality of juvenile salmon habitat. Further evidence of the importance of flow to the growth and behavior of juvenile salmon is given by the strong correlation (r = 0.92) observed between fish length and the flow velocity over the focal point of the territories of chinook salmon (Everest and Chapman 1972). In addition to the negative partial correlation between growth and flow velocity, we also observed a positive partial correlation between protein content and flow velocity. The limited differences in growth and protein levels between Rocky Brook and Sabbies River parr were attributable to the consistently higher flow conditions that prevailed in Rocky Brook.

The minimal variation of growth and body composition between parr from Sabbies River and Rocky Brook, despite significant and consistent habitat differences, suggest that *Salmo salar* (Atlantic salmon) has evolved a high degree of

homeostasis in the genetic regulation of these traits. A comparison of daily growth rates over a broader geographic area does suggest, however, that some adaptability may exist. For example, the highest daily growth rate reported for juvenile salmon in temperate regions is 0.39 mm · d^{-1} (Egglishaw and Shackley 1973), yet daily rates in northern Labrador, where the growing season is very short, may approach 0.9 mm · d^{-1} (Power 1969).

In sharp contrast to growth and body composition, the time of emigration from the nursery area and the overall body morphology of fish in the two streams differed significantly. Two principal factors may be responsible for the autumn migration of Rocky Brook large parr. First, predation rates on juvenile salmon during their passage through the lower main river and the estuary are known to be high (Elson 1962; Larsson 1977) and survivorship of fish migrating before or after the peak of migratory activity is poorer (Carlin 1968, in Osterdahl 1969). The fall movement of Rocky Brook parr may facilitate a more precise timing of their movement through the estuary, relative to the main body of smolts from other tributaries in the system. A second possible factor selecting for fall migration may be high overwinter mortality. To evaluate this hypothesis we assessed the relative severity of Rocky Brook and Sabbies River winter habitats by comparing the weight loss and changes in energy content of parr within these streams during the winter. No significant weight loss was detected between winter sampling periods in Sabbies River. In Rocky Brook, however, the elevation of the length–weight regression decreased significantly during the 1974–75 winter sampling and both elevation and slope were depressed during the 1975–76 sampling (Riddell 1979) indicating a greater weight loss in larger parr during overwintering. Daily caloric loss was greatest between October and December but did not differ between populations during this interval (Rocky Brook 13.6 J · g^{-1} · d^{-1}; Sabbies River 13.7 J · g^{-1} · d^{-1}). The period before ice formation is known to be destructive to benthic invertebrates (Hynes 1970) and is also a period of severe conditions for fish (Maciolek and Needham 1952). Between December and February, Rocky Brook parr continued to experience a net energy loss (8.0 J · g^{-1} · d^{-1}) whereas Sabbies River parr increased in energy content (4.1 J · g^{-1} · d^{-1}). Overall October–February rates of energy loss were 187–344% greater in Rocky Brook parr. The minimum difference is estimated when no net energy gain between December and February is assumed for the Sabbies River parr.

Overwintering parr in Rocky Brook (mean sample weight = 5 g) and Sabbies River (mean weight = 9 g) apparently obtain some energy from feeding even at 0–1°C. The rates of energy loss were less than the maintenance energy required per day as estimated from Elliott's (1976) equation 11 describing the relation of maintenance energy intake with wet body weight and temperature. The greater energy intake for Sabbies River parr compared to Rocky Brook parr and the estimated energy intake required to account for a 4.1 J · g^{-1} · d^{-1} increase are consistent with results of our winter feeding studies. Juvenile salmon in each stream fed actively during the winter, principally on Nemourid stoneflies, but stomachs of Sabbies River parr contained 5× the number of food items found in the stomachs of Rocky Brook parr (101 items vs. 19). If we estimate the energy content of the February benthos sample from Sabbies River (Table 2, 21.3 J · mg^{-1} dry wt), assume in situ evacuation rates (36–48 h to 100% evacuation at 0–1°C; Otto 1976; Reimers 1957), and 50% energy utilization, less than 40 Nemourid stoneflies would be sufficient to account for the daily maintenance requirements plus an average 4.1 J · g^{-1} net daily increase in energy. The severity of overwintering conditions for large parr in Rocky Brook plus the potential predator avoidance achieved by synchronized estuarine migrations could result in strong directional selection for fall migration of large parr from Rocky Brook.

The consistent differences in body and fin morphology between juvenile salmon from Rocky Brook and Sabbies River also appear related to environmental differences. Higher flow velocities may be selective for more streamlined body form and longer paired fins. The difference in fineness ratio, a measure of streamlining (body length/maximum body diameter), between Rocky Brook and Sabbies River was small (5%) and can be expected to have only a minimal effect on pressure drag (Webb 1975). The differences in fin size between populations may, however, result in significant differences in the energetic cost of maintaining feeding positions. Salmon parr are known to maintain their position on the substrate by extending their paired fins as hydrofoils. Since the lift generating ability of a hydrofoil is directly proportional to its surface area (Webb 1975), larger fins would be more effective in creating the negative lift required to maintain a station in the higher average flows characteristic of Rocky Brook.

Similar relationships of body form and fin size with flow velocity have also been reported for *Salmo trutta* (brown trout) by Yevsin (1977) and for *S. salar* by Jones (1975) and Schaffer and Elson (1975). Our findings and these supporting reports lead us to hypothesize that the differences in morphology observed are adaptive responses to the long-term flow conditions of each population's natal stream. Our hypothesis was supported by the samples collected from the three other New Brunswick streams (Fig. 2, 7). Flow velocity was an adequate index of the relative body form and fin size in populations which experienced significantly different flow velocities. Inconsistencies between LSWMR and RR comparisons suggest that other environmental parameters such as substrate roughness or riffle:pool ratios may, however, compensate for limited differences in flow velocity. Also note that the exact repeatability of the difference between population means despite small shifts in mean values between years (Fig. 6C) strongly supports a genetic basis for the interpopulation differences observed.

The importance of environmental variation in the determination of phenotypic expression varied for each trait investigated. The apparent homeostasis of growth and biochemical composition in juvenile salmon suggests that seasonal variability is more important than genetic variation in determining the expression of these traits. We believe the between-population differences in body morphology and time of downstream migration are adaptations to the rearing environments. Discriminant analyses of body morphology suggested that a minimum of 20% of the total phenotypic variance is attributable to between-population differences. The relative

contributions of genetic and environmental determinism in phenotypic traits differing between populations cannot yet be determined.

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Evidence of Adaptive Polygenic Variation Between Two Populations of Atlantic Salmon (*Salmo salar*) Native to Tributaries of the S. W. Miramichi River, N.B.[1]

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RIDDELL, B. E., W. C. LEGGETT, AND R. L. SAUNDERS. 1981. Evidence of adaptive polygenic variation between two populations of Atlantic salmon (*Salmo salar*) native to tributaries of the S. W. Miramichi River, N.B. Can. J. Fish. Aquat. Sci. 38: 321–333.

Breeding experiments were conducted to test the hypothesis that spatial homeostasis in growth and proximate composition and geographic variation in body morphology and timing of migration in juvenile Atlantic salmon (*Salmo salar*) have an adaptive basis. The populations studied were Rocky Brook and Sabbies River, tributaries of the Southwest Miramichi River, New Brunswick. Growth rates and proximate composition were very similar in the two populations under hatchery conditions. Genetic contributions to homeostasis in these traits were largely additive. The phenotypic expression of migratory behavior and the genetic control of timing were not quantifiable. A genetic basis to interpopulation variation in body morphology was demonstrated. Progeny from Rocky Brook which exhibits higher flows had more fusiform bodies and longer fins than progeny from Sabbies River when reared under identical conditions. Heritable variation in morphology and a demonstrated directional selection for greater fin size in high velocity environments support the hypothesis that these traits are adaptive. Adaptive polygenic variation between local populations stresses the need to delineate stocks on a functional basis so that species can be managed without significant loss of fitness and/or depletion of genetic variation in natural populations.

Key words: quantitative genetics, heritability, adaptation, morphology, growth, proximate composition, migration, Atlantic salmon, interdemic variation, directional selection

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Les expériences d'élevage décrites dans l'article qui suit furent entreprises en vue de vérifier l'hypothèse que l'homéostasie spatiale dans la croissance et la composition par catégories de constituants, d'une part, et dans la variation géographique de la morphologie du corps et de l'époque de la migration chez les jeunes saumons atlantiques (*Salmo salar*), d'autre part, a un fondement d'adaptation. Nous avons étudié les populations du ruisseau Rocky et de la rivière Sabbies, tributaires de la rivière Miramichi sud-ouest, Nouveau Brunswick. Dans des conditions d'élevage en établissement de pisciculture, le taux de croissance et la composition par catégories de constituants se ressemblent beaucoup chez les deux populations. Les contributions génétiques à l'homéostasie quant à ces traits sont en grande partie cumulatives. Nous n'avons pu quantifier l'expression phénotypique du comportement migrateur, non plus que le contrôle génétique de l'époque de l'avalaison. Nous avons pu démontrer un fondement génétique à la variation interpopulation de la morphologie du corps. Les descendants des saumons du ruisseau Rocky, dont l'écoulement est plus fort, ont le corps plus fusiforme et des nageoires plus longues que ceux de la rivière Sabbies, lorsque ces poissons sont soumis à des conditions d'élevage identiques. Cette variation de morpholo-

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gie transmissible ainsi qu'une sélection en faveur de plus grandes nageoires dans des environnements de grande vélocité corroborent l'hypothèse que ces traits ont une valeur d'adaptation. Une variation phylogénique d'adaptation entre des populations locales fait ressortir le besoin de définir les stocks sur base fonctionnelle, de sorte que l'on puisse gérer les espèces sans détérioration importante de l'état physique ou diminution de variation génétique chez les populations naturelles, ou les deux.

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ELECTROPHORETIC evidence of genetic variation between natural populations has been reported for a wide variety of organisms (Nevo 1978) and provides convincing support for Ehrlich and Raven's (1969) hypothesis that the local population is the unit within which evolution occurs. The potential importance of genetic variation to the management of fish populations was quickly identified, particularly for salmonids (de Ligney 1971; Møller 1970). While the potential usefulness of electrophoretic studies to fisheries research and management is unquestionable, they do not, by themselves, constitute a sufficient basis for the definition of management units or strategies in natural populations. This is, in part, because genetic variation has been detected between very localized populations (Hedgecock 1978; Highton 1977; Møller 1970). Consequently, as the number of electrophoretically identifiable stocks increase, their individual management becomes increasingly impractical. Furthermore, a lack of electrophoretically detectable genetic variation between populations does not necessarily imply a lack of phenotypic variation, the correlation between genetic and phenotypic variances being uncertain (Soulé et al. 1973; Thompson 1975; Highton 1977; Sokal 1978; Ryman et al. 1979). There continues to be a need for a greater understanding of the relationship between spatial and temporal variation in genetic and phenotypic traits (Smith et al. 1976; Hedgecock et al. 1976).

In a recent paper (Riddell and Leggett 1981) we reported the occurrence of significant differences in body morphology and time of downstream migration in juvenile salmon native to two tributaries of the Miramichi River, New Brunswick. These differences appeared to be determined by differences in temperature and flow regimes in the natal streams and were hypothesized to be adaptive. Partial confirmation of this hypothesis was achieved when interpopulation differences in body morphology of juvenile salmon in other Atlantic coast rivers were successfully predicted a priori from a knowledge of flow characteristics. In this paper we report the results of a further quantitative genetic test of our hypothesis using breeding experiments to demonstrate that observed differences in body morphology and migration characteristics in juvenile Atlantic Salmon have a genetic basis.

Methods

EXPERIMENTAL DESIGNS AND DATA COLLECTION

Two years of controlled breeding experiments were conducted to investigate the genetic basis of phenotypic differences observed between natural populations of salmon parr from Sabbies River and Rocky Brook, tributaries of the Main Southwest Miramichi River. Fish were reared at the North American Salmon Research Center in St. Andrews, New Brunswick.

In 1975, three replicate rearing groups were created from each population. Each replicate consisted of one-third of the pooled eggs from six families (three females × two males) per population. The eggs in each rearing group were hatched in separate incubation trays and the fry of each group were transferred at swim-up to separate 1.97-m^2 circular Fiberglass tanks with peripheral flows of 0.25 ± 0.06 m · s^{-1}. Tank water capacity was 160 L and water replacement time was ~ 6 h. During incubation and early growth periods the water was heated (Fig. 1) and oxygen levels were rarely less than 95% air saturation. Fry densities were standardized to the number of fry in the rearing group with the poorest survival to swim-up. Survival was generally poor throughout the hatchery during 1975–76. Survival to swim-up in Rocky Brook replicates was 24–52% and 12–21% in Sabbies River replicates. Mortalities forced the termination of this experiment 15 wk after swim-up. Length and weight were measured on 25 fish in each rearing group at swim-up and subsequently at 4-wk intervals until termination. Nine morphometric characters: head depth (HD), head width (HW), maximum body depth (MBD), maximum body width (MBW), head length (HL), anterior of snout to insertion of pectoral fin (SNAP), pectoral fin length (PL), anterior of snout to insertion of pelvic fin (SNAPE), and pelvic fin length (PEL) were measured at the end of 4 and 15 wk. These characters conformed to those analyzed in our earlier study of interpopulation differences in the phenotypic characteristics of wild populations (Riddell and Leggett 1981). A nested analysis of variance was used to partition the variation between replicates within populations and the variation between populations (Riddell 1979).

In 1976–77, a factorial breeding design within populations was used to define the genetic control of growth and body morphology. The number of available tanks allowed for 12 families per population (four females × three males). The death of one male before the completion of the Sabbies River crosses necessitated the use of a fourth male to produce 12 families from this popualtion. The water was again heated during incubation and early development (Fig. 1). Fry were transferred to individual rearing tanks at swim-up. Mortalities during the commencement of feeding were high, as in 1975–76, but by early May mortality rates were very low and fry densities were then decreased to a maximum of 200 individuals per tank by random removal. When the parr were ~ 7.5 cm in length (July 25) the rearing density of the indoor tanks was decreased to 100 individuals per tank.

Length, weight, head length (HL), pectoral fin (PL), and pelvic fin length (PEL) were measured on 25 individuals per

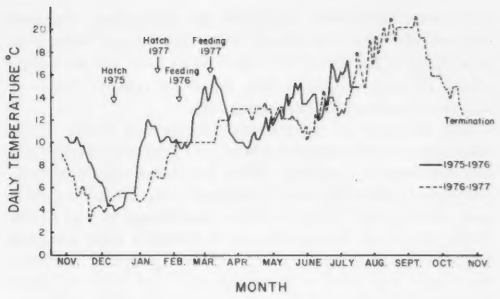

FIG. 1. Daily temperature during the two years of controlled breeding experiments.

family each month beginning at swim-up. At 3-mo intervals, 25 parr from each family were sacrificed for proximate analysis and measurement of all nine morphometric features. All sacrificed fish were frozen on dry ice and stored at −40°C preceding analysis.

In preparation for proximate analysis, all individuals were dried to constant weight at 80°C and ground to a find powder. Moisture was estimated by wet weight loss, and protein and ash were assayed as described in Riddell and Leggett (1981). Triplicate protein and duplicate ash determinations were conducted. Lipid content was determined by substraction. The accuracy of lipid estimates was periodically evaluated by extraction of lipids from dried tissues by refluxing with 100% chloroform for 6 h.

Standardized discriminant functions, without data transformation to a uniform fork length, were used to determine whether the between-population morphological variation observed in natural populations (Riddell and Leggett 1981) persisted under controlled rearing conditions. Comparisons between replicates within populations and between populations were possible during the 1975−76 experiment and further subdivisions within populations were possible during the 1976−77 experiment. SPSS program Discrimination (Nei et al. 1976) was utilized in this analysis. All other programs utilized were from SPSS or SAS (Barr et al. 1976) libraries.

Growth rate analyses during 1976−77 breeding experiment utilized Krüger's (1973) "Reziprokfunktion." The linearized form of this function is:

$$\log y = \log Y\infty - \frac{1}{x + \epsilon} \cdot \log N$$

where $Y\infty$ = maximum length,
N = growth rate constant,
x = age calculated from birth (postnatal),
ϵ = additive age term (prenatal).

This function is characterized by an inflection point, necessary to describe the growth form of juvenile fishes, and corresponds closely to the more widely used Gompertz and Pütter−Bertalanffy equations (Krüger 1973). The additive age term ϵ mathematically defines the shape of the curve and need not correspond to actual prenatal ages; however, in 18 of 23 growth regressions estimated for our data, the actual prenatal age improved the fit of the estimated regressions. The true prenatal age was consequently incorporated in all growth equations. When the additive age term is the same in each growth curve, valid comparisons of growth rate can be made (Krüger 1973).

Surplus fry from eight rearing groups (four families per population) were used to investigate genetic regulation of the time of downstream migration. One hundred individuals from each of the eight rearing groups were randomly collected and finclipped to identify families. Families were then randomly assigned to two 90-m^2-circular outdoor tanks. Between-tank variation was evaluated by finclipping a replicate sample from one family in each population and assigning these replicates to the opposite tank from which the family was initially assigned.

The level of circulating thyroxine was used as an index of the migratory condition of parr. Changes in thyroid activity have been associated with smoltification and migration (Hoar 1976) and with migratory activity alone (Simpson and Youngson 1979). Thyroxine sampling was conducted in mid-November, when water temperatures at the hatchery decreased to the ambient temperatures associated with downstream migration in Rocky Brook. Blood was withdrawn from the caudal vessel with a heparinized tuberculin syringe following anesthesia with MS222; 10 fish were sampled from each rearing group. The plasma was separated by centrifugation and plasma from each individual was split into two 150-μL samples; each ampoule was then lyophilized for storage. Thyroxine and triiodo-L-thyroxine were analyzed by radioimmunoassay (Brown and Eales 1977).

STATISTICAL AND GENETIC MODELS

Genetic variance components for the 1976−77 breeding design were estimated using a two-way cross-classified analysis of covariance.

$$Yijk = u + Di + Sj + (s \times d)\,ij + bZijk + eijk$$

where $Yijk$ = the observation of the kth individual with dam i and sire j,
u = theoretical population mean,
Di = effect of ith dam,
Sj = effect of jth sire,
$(s \times d)ij$ = interaction effects of ith dam × jth sire full-sib group,
$Zijk$ = covariate effect assuming equal slope (b) between families (i = 1,2,3,4; j = 1,2,3),
$eijk$ = effect of individuals within family (i,j); random error.

The assumptions of the model were; (1) $Yijk$ are random variables distributed about a common mean u; (2) the components of the model combine additively, have zero mean and covariances, and are normally distributed with variances σ_d^2, σ_s^2, $\sigma_{s \times d}^2$, σ_e^2. The expected genetic composition of the statistical variance components, ignoring interactions higher

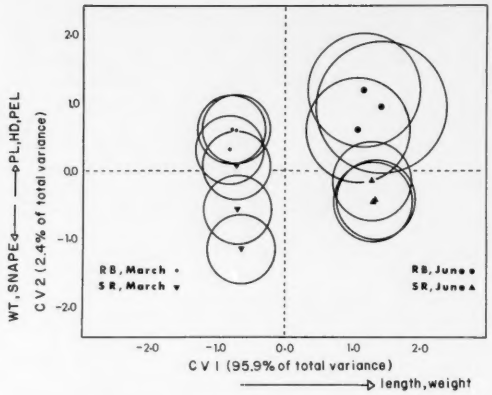

FIG. 2. Discriminant function analysis of 1975–76 controlled breeding experiments. Circles about each replicate's centroid are the 95% confidence area as estimated by Seal (1964). The function for each axis is:
Canonical variate (1) $Y = 1.22\,LT - 0.65\,WT + 0.2\,HD + 0.38\,HW + 0.24\,HL - 0.06\,SNAP - 0.26\,PL - 0.11\,PEL$ $R^2 = 0.98$
Canonical variate (2) $Y = 0.62\,LT - 2.0\,WT + 1.42\,HD - 0.2\,HW + 0.0\,HL - 2.5\,SNAP + 1.71\,PL + 1.11\,PEL$ $R^2 = 0.63$
R^2 = canonical correlation, proportion of variation accounted for by the function which is attributable to between-group variation.

than two loci, is:

Sire component	$\sigma_s^2 = 1/4\,\sigma_{10}^2 + 1/16\,\sigma_{20}^2$
Dam component	$\sigma_d^2 = 1/4\,\sigma_{10}^2 + 1/16\,\sigma_{20}^2 + \sigma_M^2$
Interaction component	$\sigma_{s\times d}^2 = 1/4\,\sigma_{01}^2 + 1/8\,\sigma_{20}^2 + 1/8\,\sigma_{11}^2 + 1/16\,\sigma_{02}^2 + \sigma_T^2$
Error	$\sigma_e^2 = 1/2\,\sigma_{10}^2 + 3/4\,\sigma_{01}^2 + 3/4\,\sigma_{20}^2 + 7/8\,\sigma_{11}^2 + 15/16\,\sigma_{02}^2 + \sigma_e^2$

where σ_{10}^2 describes single gene additive variance; σ_{20}^2 describes nonallelic pair additivity; σ_{01}^2 describes allelic pair interaction (dominance variance); σ_{11}^2 describes allelic pair × single gene interactions (epistasis), and σ_M^2 describes maternal genetic plus maternal environmental effects (Becker 1975).

Heritability, a measure of additive genetic effects which are common by descent in a population, was estimated from the sire components. Dominance estimates ($4 \times \sigma_{s\times d}^2$) overestimate dominance effects since the estimator contains limited nonallelic pair interaction, epistasis, and is confounded with between-tank variation (σ_T^2). Phenotypic, genetic, and environmental correlations between all possible pairs of variables (length, weight, head length, pectoral fin and pelvic fin lengths) were also evaluated. Approximations to the standard errors of heritability and genetic correlation estimates were derived using formulas outlined by Robertson (1959a, b).

We utilized the SAS, GLM program (Barr et al. 1976) to account for covariants (length or age) and to derive reductions due to dam, sire, and interaction terms. Variance components for sire, dam, and interaction were estimated by Henderson's method 3 (Henderson 1953; Searle 1971). However, since survival was unequal between families the reductions calculated in an unbalanced design are not orthogonal. Variance components were calculated using the mixed reductions procedures of Harville (1967) and Low (1964), and the significance of each reduction was tested by type IV sums of squares (Searle and Henderson 1978).

The adequacy of the additive–dominance model in the regulation of body morphology and the generality of the relative importance of additive versus dominance effects on morphological parameters were evaluated using Cavalli's (1952) joint scaling test. Twelve families maintained as part of the North American Salmon Research Center's main crossing program were utilized (three pure Rocky Brook families, three pure Big Salmon River families, and their pair-wise maternal and paternal hybrids). All nine morphological parameters were measured on 50 fish per family. The measurements were then standardized within each family to the overall grand sample mean length (Thorpe 1976). A discriminant function was evaluated between the pure Rocky Brook and pure Big Salmon River families and the value of the function subsequently evaluated for each hybrid individual. The scaling test was then evaluated for family mean discriminant values weighted by the reciprocal of the variance about each family mean.

Results

PRELIMINARY EXPERIMENTS 1975–76

The similarity of growth rates and the differences in morphological traits observed between Rocky Brook and Sabbies River parr under natural conditions (Riddell and Leggett 1981) persisted in controlled rearing environments. Our confidence in these results was, however, diminished because of poor survivorship. Rocky Brook fry were larger at swim-up than were Sabbies River fry (Rocky Brook 2.38 ± 0.07 cm; Sabbies River 2.35 ± 0.07 cm, t-test, $n = 75$, $P < 0.01$). Growth rates and weight–length relationships after swim-up, however, did not differ significantly between populations. Between-tank differences in length accounted for less than 1% of the total variation until 10 wk after swim-up, but increased to 8% by the termination of the experiment.

Morphological variation was consistent with differences observed in the natural populations (Riddell and Leggett 1981). The first axis of Fig. 2 is size-related, separating March and final June samples. The second axis, however, discriminates between populations based upon an inverse relationship between weight and a group of variables (head depth, pectoral and pelvic fin length). Individuals in Rocky Brook replicates were less robust, had greater head depth, and longer pectoral and pelvic fins. Morphological differences between populations were not significant for the pooled March samples ($P > 0.05$). The distance between population means increased during growth and was significant by June ($d = 1.51$, $P < 0.01$). While it could be argued that the high mortalities experienced during larval growth were selective, we believe that the increasing morphological variation between populations was indicative of differences in ontogenetic development. Ninety-five percent of the mortalities occurred before the March comparison and yet no significant morphological differences were detected in the March sam-

TABLE 1. T-test comparisons of $H_o : \bar{x}_{RB} = \bar{x}_{SR}$ for egg volume (mL), length and weight, and pectoral fin length at swim-up (mean ± 1 SD).

	Sabbies River (n = 12)	Rocky Brook (n = 11)	P
Length of female parents	(71.0−88.5 cm)	(68.0−75.0 cm)	
Egg volume	0.132±0.015	0.168±0.011	<0.01
Length (cm)	2.56±0.064	2.61±0.079	>0.05
Weight (g)	0.116±0.022	0.134±0.013	<0.05
Pectoral fin length (cm)	0.50±0.029	0.558±0.026	<0.05

ples. Further, while growth rates were equal in the two populations, Rocky Brook parr had longer paired fins. At equal sample mean lengths in June, the pectoral fin lengths were Sabbies River parr 1.09 ± 0.129 cm (n = 60) and Rocky Brook parr 1.14 ± 0.119 cm (n = 26) (t-test = 1.9, $0.05 < P < 0.01$).

GENETICS EXPERIMENT 1976−77

Egg and early fry survival — Hatching time (80−81 d) and survival to hatching (97.6 ± 0.87%) were similar in all families except one, a Rocky Brook family which suffered 100% egg mortality. Survival decreased slightly during alevin development (80 ± 11.2%) and was strongly dependent upon the female parent. The maternal variance component for the $\sin^{-1}$ (% survival)$^{0.5}$ to swim-up, derived from a two-way ANOVA without interaction (the full model defined in the methods was untestable in the Rocky Brook crosses because of the loss of one family), accounted for 62 and 72% of the total variance in Rocky Brook and Sabbies River families, respectively. Survival decreased substantially and maternal dependence became increasingly obvious after exogenous feeding began. Survival at 1 m after the commencement of feeding was 67.4 ± 15.9%. Survival was generally greater in Rocky Brook families and may be related to the significantly larger egg size of Rocky Brook females and the resultant greater average alevin length, weight, and pectoral fin length in this population (Table 1). Peterson (1975) noted the importance of pectoral fin movements to the effective clearing of the posterior opercular margins of Atlantic salmon alevins. The larger pectoral fins may assist alevins in the clearance of debris common in hatchery tanks during the commencement of feeding.

Growth rate and weight−length relationships — Growth rates were similar between all families although some evidence of significant sire effects was observed. The slope of the weight-length regressions were greater in Rocky Brook families relative to Sabbies River families. Two difficulties were encountered while analyzing growth rates. Inclusion of the length at swim-up in growth rate regressions reduced the fit of the regressions. Since body size at swim-up is determined by larval development, these data were omitted from growth rate analyses and were subsequently analyzed separately. Secondly, rearing densities significantly affected growth rate and weight−length relationships (correlations of tank density with tank mean lengths and weights at the termination of the experiment were −0.49 and −0.75, respectively). Therefore, only families with equal rearing densities (±10%) were included in the growth analyses.

Length and weight of swim-up fry were greater in Rocky Brook families than in Sabbies River families (Table 1). Maternal effects (maternal genetic plus maternal environmental effects, Willham 1972) and additive effects influenced the expression of both traits (Table 2A). Maternal effects were most prevalent in the expression of weight variation and within the Sabbies River population. Sire estimations of heritabilities were, therefore, used for this variable and population. A pooled estimate for length at swim-up in Rocky Brook is the preferred estimation, however $(2[\sigma_s^2 + \sigma_d^2]/\sigma_P^2 = h^2 = 0.40 \pm 0.11$; Becker 1975). Significant interaction and sire effects for length at swim-up in Rocky Brook (Table 2A) suggests paternal contributions in the regulation of alevin and fry development in this population.

Within-population variation in growth rates (caluclated from 165 d post fertilization onward, Fig. 3A) was observed, but variation between populations was not significant (F ratio test, P = 0.86). Between-population comparisons of the weight−length relationship reveal a significantly greater slope within the Rocky Brook population (F ratio test, P = 0.006). Mean regressions within each population were:

$$\text{Rocky Brook WT} = 0.0069\ \text{LT}^{3.20} \qquad R^2 = 0.99$$
$$\log_{10} Sy{\cdot}x = 0.0628 \qquad n = 1052$$
$$\text{Sabbies River WT} = 0.0074\ \text{LT}^{3.17} \qquad R^2 = 0.99$$
$$\log_{10} Sy{\cdot}x = 0.0477 \qquad n = 1418.$$

Heritabilities of growth rate in Sabbies River and of the weight−length relationships in both populations were consistently less than 0.05. The heritability of growth rate in the Rocky Brook population was higher (pooled sire plus dam estimates of $h^2 = 0.194 \pm 0.077$). A sire influence on Rocky Brook growth rates is suggested in Fig. 3A, the two families with the lower growth rates (families 4, 5) being paternal half-sibs. Maternal effects remained important genetic components in the growth of Sabbies River families, but were insignificant after swim-up in Rocky Brook families. Prolonged maternal effects may have been maintained by consistently greater growth rates of the larger fry (Gall 1974).

Body morphology — As observed during the 1975−76 experiment, body morphometry was similar (all families pooled) at small body sizes, but diverged continuously as body size increased. At the termination of the experiment, body morphometry was significantly different between populations (d = 1.23, F ratio test, $P < 0.001$). The increasing morphological divergence during growth is obvious from Fig. 4, in which the third axis consistently differentiates between Sabbies River and Rocky Brook parr. This separation is based upon the more robust body form and smaller pectoral

TABLE 2. ANOVA of Rocky Brook and Sabbies River fry length and weight at swim-up (A) and length−age, weight−length, allometric and fat-free dry matter (protein + ash) to length relationships between 165 d of age and the termination of the experiment (B). Heritabilities are derived from the statistical variance components presented, significance levels of the variance components result from tests of the ANOVA mean squares, test procedures for B are described in the methods. *($P < 0.05$.)

		Rocky Brook				Sabbies River			
A.		Dam	Sire	Interaction	Residual	Dam	Sire	Interaction	Residual
	df	3	2	5	264	3	3	5	288
Length	$\hat{\sigma}^2$	0.0016*	0.0014*	0.001*	0.0035	3.5×10^{-4}	6.8×10^{-5}	4.4×10^{-5}	1.4×10^{-4}
	h^2	0.03	0.73(±0.13)			0.47	0.45(±0.05)		
Weight	$\hat{\sigma}^2$	$1.2 \times 10^{-4*}$	$2.5 \times 10^{-5*}$	$5.8 \times 10^{-5*}$	9.5×10^{-5}	$8.4 \times 10^{-3*}$	1.2×10^{-3}	3.4×10^{-3}	1.6×10^{-3}
	h^2	0.31	0.34(±0.18)			0.63	0.41(±0.05)		
B.		Rocky Brook				Sabbies River			
Growth Rate (lt vs. age)	df	2	2	1	1047	2	3	3	1408
	$\hat{\sigma}^2$	$9.5 \times 10^{-5*}$	$3.4 \times 10^{-4*}$	-1.2×10^{-5}	4×10^{-3}	$1.9 \times 10^{-4*}$	1.4×10^{-5}	4×10^{-5}	3.8×10^{-3}
	h^2	−0.05	0.30(±0.1)			0.02	0.014(±0.018)		
Weight vs. Length	df	2	2	1	1047	2	3	3	1408
	$\hat{\sigma}^2$	-4.8×10^{-5}	-4.7×10^{-5}	$6.5 \times 10^{-5*}$	4.2×10^{-3}	$1.1 \times 10^{-4*}$	-5.2×10^{-6}	2.4×10^{-5}	2.2×10^{-3}
	h^2	$2(\hat{\sigma}_d^2+\hat{\sigma}_s^2)/\hat{\sigma}_P^2 = -0.05(\pm 0.03)$				0.04	−0.01(±0.1)		
Head length	df	3	2	5	1513	3	3	5	1788
	σ^2	$1.09 \times 10^{-5*}$	3.08×10^{-6}	2.3×10^{-6}	4.4×10^{-3}	$1.16 \times 10^{-5*}$	-0.65×10^{-6}	$1.48 \times 10^{-5*}$	2.78×10^{-4}
	h^2	0.06	0.03(±0.025)			0.036	−0.01(±0.024)		
Pectoral fin lt	df	3	2	5	1513	3	3	5	1788
	σ^2	$1.3 \times 10^{-5*}$	0.8×10^{-5}	$2.1 \times 10^{-5*}$	7.6×10^{-4}	$1.6 \times 10^{-4*}$	$6.38 \times 10^{-5*}$	$5.13 \times 10^{-5*}$	7.76×10^{-4}
	h^2	0.053	0.04(±0.029)			0.42	0.24(±0.062)		
Pelvic fin lt	df	3	2	5	1513	3	3	5	1788
	σ^2	$4.6 \times 10^{-5*}$	0.66×10^{-5}	$1.73 \times 10^{-5*}$	1.09×10^{-3}	$2.61 \times 10^{-5*}$	$1.06 \times 10^{-5*}$	$5.86 \times 10^{-5*}$	1.19×10^{-3}
	h^2	0.091	0.022(±0.022)			0.057	0.033(±0.023)		
FFDM	df	1	2	1	219	2	2	3	351
	σ^2	4.42×10^{-5}	$5.03 \times 10^{-3*}$	-2.5×10^{-5}	1.14×10^{-3}	$2.6 \times 10^{-4*}$	1.63×10^{-5}	-3.05×10^{-5}	9.94×10^{-4}
	h^2	0.156	0.166(±0.154)			0.45	0.052(±0.068)		

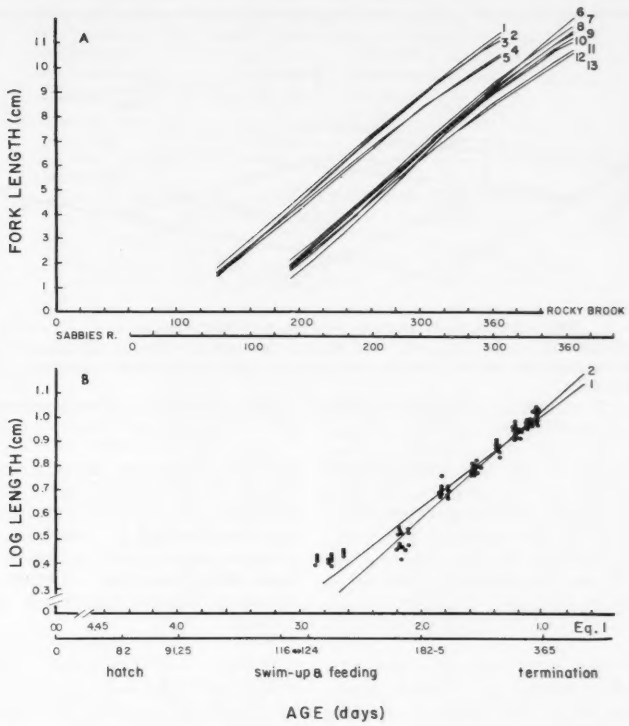

FIG. 3. Growth rate of Atlantic salmon parr from swim-up to 355 d after fertilization. A. Family growth curves for 13 families with equal rearing densities, x axes are equally scaled but shifted to separate populations. B. Regression of mean growth rate for all parr measured in the 13 families used in (A). Fertilization = day zero and in the linearized Krüger growth functions X = days of age after hatch at day 82. Equation (1).

$$\log y = 1.3995 - \frac{0.3811}{(x \div 365) + 0.2247} \qquad R^2 = 0.91\ Sy \cdot x = 0.067\ n = 104$$

Equation (2) estimated without fry size at swim-up included.

$$\log y = 1.5068 - \frac{0.4619}{(x \div 365) + 0.2247} \qquad R^2 = 0.98\ Sy \cdot x = 0.027\ n = 91$$

fin lengths of Sabbies River parr (Rocky Brook pectoral fin length = 1.91 ± 0.280 cm, Sabbies River PL = 1.84 ± 0.273; t-test, $P \leq 0.05$). Pectoral and pelvic fin lengths and head form have consistently been the principal variables in the separation of Rocky Brook and Sabbies River populations, both under natural (Riddell and Leggett 1981) and controlled rearing conditions. Slopes of the allometric equations for pectoral and pelvic fin length varied between families and were significantly greater in Rocky Brook families during the 1976–77 growth experiment. Variation in head length was more limited and the slopes of the allometric regression for head length were equal between populations (Table 3). The genetic basis of variations about the mean allometric relation of fin and head length were similar between traits and between populations, except in one instance (Table 2B). Dominance estimates for the head and fin length traits averaged 12% of the phenotypic variance (range of 2–18%), but these values may be inflated by tank effects. Levels of additive variance were smaller, averaging 6% (range from 2 to 24% of the phenotypic variance).

Although there is considerable variation in the relative importance of the statistical variance components in Table 2, dominance effects were generally most prevalent. The adequacy of the additive-dominance model in explaining the general body morphology of a family was tested by analyzing hybrid crosses as outlined in the methods. The family mean discriminant values (±1 SD) are given in Table 4. A scaling test applied to these family means assumes an absence of significant reciprocal effects. Replicate 3 demonstrates marked reciprocal effects and was omitted from the scaling

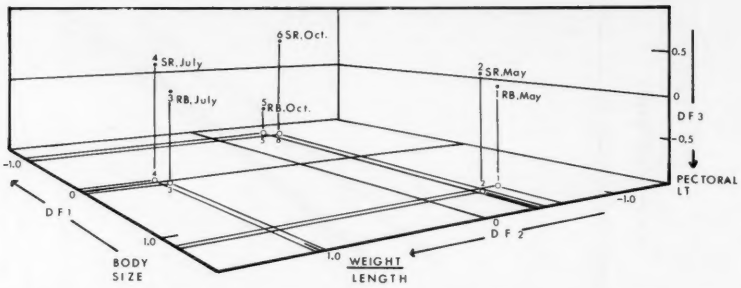

FIG. 4. Body morphology comparisons between populations during 1976–77 growth experiment. Increasing body size during growth is accounted for by canonical variate 1, and variate 2 was dependent upon weight : length variation between sampling periods. Between-population variation is accounted for by 3rd variate:

$$CV\ 3 = 0.94\ LT - 0.67\ Wt - 1.8\ HD - 1.4\ HW + 4.7\ MBW - 1.3\ MBD + 2.5\ HL - 3.7\ PL + 0.6\ PEL \quad R^2 = 0.37.$$

test. The results of the scaling test reflect the standardizations performed on the data previous to the analysis, but would indicate that the additive-dominance model is adequate in this simple analysis of family means (Chi-square test, $0.10 < P < 0.20$). Body morphology of Big Salmon River fish was dominant to that of Rocky Brook. The parameters estimated in Cavalli's (1952) joint scaling test were: $\hat{m} = -0.04 \pm 0.27$, $\hat{d} = 0.79 \pm 0.27$, $\hat{h} = 0.54 \pm 0.41$, the degree of dominance ($\hat{h}/\hat{d}$) being 0.68.

The ability to discriminate significantly between Rocky Brook and Big Salmon River parr and then to define a model adequately describing the genetic mean of the hybrid family clearly indicates genetic variation in body morphology exists between populations.

Genetic and environmental correlations — The similar relative magnitudes of the variance components for length, weight, and the morphometric features were accounted for by the strong genetic correlations between these variables (Table 5). Since the sum of the genetic variance components was generally small relative to the environmental variance (a fact that accounts for the small number of negative variance estimates), we estimated the genetic correlations by pooling all data, regardless of population of origin. A two-way ANOVA without interaction was used to estimate the variance components.

Howells (1972) has argued that observed patterns of variation between populations result from a limited number of complexes of covarying traits within individuals. In this connection it is significant that the smallest genetic correlations observed in our data were between the length of the pectoral and pelvic fins and body length. Selection could then operate on fin size with limited independence from body length and other morphological traits more highly correlated with body length. Howells' suggestion is further supported by the results of a principal factor analysis (SPSS factor, Nei et al. 1975) of the complete morphometric data set, collected during October 1977 and separated by population. After controlling for length and weight, which accounted for 53–58% of the phenotypic variance, the structure of the residual correlation matrix could be partitioned into three factors accounting for 68 ± 1% of the residual variation (Riddell 1979). In both populations morphological traits consistently grouped as follows: one factor describing head and body width and depth, a second factor comprised only of pectoral and pelvic fin lengths, and a third factor, with an eigenvalue just greater than 1, which indicated covariation between head length and position of girdle insertions.

Proximate body composition — The relationships between protein, ash weight, body length and/or weight did not differ significantly between Rocky Brook and Sabbies River families. Since no significant differences in the slope of any proximate body constituent with length occurred between populations, only mean regressions for the data are reported (Table 6). Genetic variance components accounting for the rate of increase in fat-free dry matter (FFDM = ash + protein weights), which constitutes the structural material for growth, reflect the dam and sire components accounting for growth rate (Table 2).

Timing of downstream migration — We found no evidence of increased thyroxine levels in large Rocky Brook parr sampled in the fall at the time of downstream movement in wild fish. Thyroxine and triiodo-L-thyronine levels in the parr were highly variable, no significant differences were detected between populations. Levels of thyroxine and thyronine ($x \pm 1$ SD) at each population's mean length (±1 SD) were 47.8 ± 41.0 and 32.3 ± 21.4 ng/100 mL plasma in Rocky Brook families (mean length 16.1 ± 2.4 cm) and 61.4 ± 46.6 and 34.5 ± 9.6 ng/100 mL plasma in Sabbies River families (mean length 17.2 ± 1.4 cm).

Discussion

Wild Atlantic salmon parr from Rocky Brook are more streamlined in general body form and possess relatively larger pectoral and pelvic fins than do parr from Sabbies River (Riddell and Leggett 1981). Pure Rocky Brook and Sabbies

TABLE 3. Allometric equations for head length, pectoral and pelvic fin length estimated from pooled families within each population. Regression standard error ($Sy \cdot x$) is in $\log_{10}$ units, S_b = standard error of slope coefficient and F ratio tests $H_a : b_{RB} = b_{SR}$, df = 22 451 from an analysis of covariance. Allometric regressions were estimated over the length range 2.5−12.5 cm.

Trait	n	Equation	r^2	$Sy{\cdot}x$	Sb	P
Head Length						
Rocky Brook	1047	$HL = 0.2774\ LT^{0.895}$	0.98	0.021	0.004	>0.05
Sabbies River	1408	$HL = 0.2750\ LT^{0.898}$	0.99	0.018	0.003	
Pectoral Length						
Rocky Brook	1047	$PL = 0.245\ LT^{0.860}$	0.97	0.028	0.005	<0.001
Sabbies River	1408	$PL = 0.250\ LT^{0.800}$	0.96	0.031	0.004	
Pelvic Length						
Rocky Brook	1047	$PEL = 0.151\ LT^{0.953}$	0.96	0.034	0.006	<0.001
Sabbies River	1408	$PEL = 0.150\ LT^{0.938}$	0.97	0.032	0.004	

TABLE 4. Family mean discriminant value ±1 SE. Hybrid families within each row are paternal and maternal reciprocal crosses by sires and dams in the same row. RB = Rocky Brook, BS = Big Salmon, s = sire, d = dam.

	$RB_s \times RB_d$	$RB_s \times BS_d$	$BS_s \times RB_d$	$BS_s \times BS_d$
Replicate 1	−1.23±0.56	0.09±0.57	0.34±0.66	0.75±0.53
Replicate 2	−0.50±0.52	0.83±0.62	0.72±0.56	0.75±0.57
Replicate 3	−0.61±0.53	1.07±0.57	−0.30±0.57	0.89±0.54

TABLE 5. Genetic (above diagonal) and environmental correlations and heritability estimates from the pooled population data. Phenotypic correlations can be related to these components by $r_P = r_g\ (h_x^2\ h_y^2)^{1/2} - r_e.\ [(1 - h_x^2)\ (1 - h_y^2)]^{1/2}$. Estimates of the coefficient of variation of the genetic correlations were less than 1%.

	Length (cm)	Weight (g)	Head length	Pectoral fin lt	Pelvic fin lt
Length $h^2 = 0.020$		1.0	0.999	0.954	0.970
Weight $h^2 = 0.0207$	0.996		0.997	0.954	0.968
Head length $h^2 = 0.0193$	0.995	0.993		0.959	0.961
Pectoral fin length $h^2 = 0.0261$	0.987	0.987	0.988		0.914
Pelvic fin length $h^2 = 0.0138$	0.988	0.989	0.988	0.987	

River families reared from the egg stage to large parr under controlled conditions in two separate experiments maintained these interpopulation differences. The gradient and flow velocity of Rocky Brook are greater than in Sabbies River; we therefore interpreted these results to be strong support for our hypothesis that these differences represent adaptations to natal rearing environments (Riddell and Leggett 1981).

This interpretation of our results is made in full appreciation that genetic and rearing environments are confounding on interfamily differences when reared groups do not share identical environments. Between-tank variation was limited, however, and effects due to this error source were minimized by comparing families which had equal rearing densities. The range of phenotypic variation accounted for by between-tank variation in our studies (1−8%) was consistent with the 2−7% range reported by Refstie et al. (1977) and Aulstad et al. (1972). Between-tank variation was confounded with dominance in the estimates of genetic sources of variation; however, the average dominance estimate in our study (9.6% of the phenotypic variance, range 0−18%) is also consistent with the 7% estimate of Refstie et al. (1977). Tank effects appear to have been limited but replication of families between tanks would have been necessary to more carefully quantify between-tank variance in the 1976−77 experiment. Maternal variance components similarly contain unquantified sources of phenotypic variance. Maternal environmental effects on egg and larval survival of fishes depend upon body size, age, egg size, and quality (Gall 1974; Nikolskii 1969). The partitioning of maternal effects between genetic and environmental components is not clearly understood. Our study results and corroboration from the literature suggests that the expression of maternal genetic effects are largely limited to egg and, to a lesser degree, early larval stages. Maternal effects accounted for 41−86% of the variability in egg viability (Kanis et al. 1976; Ayles 1974) and 62−72% of the variance in survival to swim-up in this study. A further potential source of error in our interpretation is the assumption that genotype−environment interactions were equal between families. This assumption is considered realistic for first generation crosses of wild stocks reared under controlled conditions.

The contribution of the combined genetic variances to the residual variation about the population mean growth rate (Fig. 3) and the allometric equations for weight, head length, pectoral and pelvic fin length, subsequent to swim-up, were consistently less than 20% and were generally lower in the

TABLE 6. Body weight and proximate body composition to body length regressions for all individuals analyzed (n = 585). $Sy \cdot x$ = regression standard error in $\log_{10}$ units, S_b = standard error of slope exponent.

Regression	r^2	$Sy \cdot x$	s_b
WT = 0.01 $LT^{3.04}$	0.995	0.032	0.010
Protein wt = 0.0011 $LT^{3.21}$	0.995	0.036	0.010
Ash wt = 1.5×10^{-3} $LT^{3.22}$	0.993	0.041	0.012
Ash wt = 0.1477 protein $wt^{1.00}$	0.993	0.038	0.003
FFDM = 1.25×10^{-3} $LT^{3.21}$	0.995	0.032	0.010

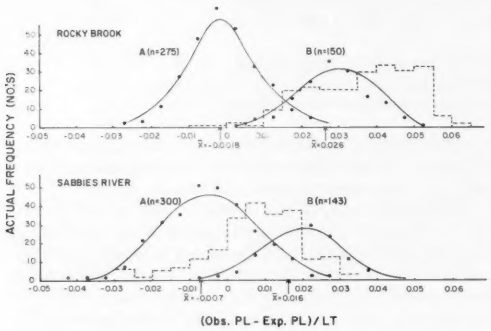

FIG. 5. Frequency distribution of (observed PL − expected PL)/fork length for hatchery-reared swim-up fry (A), wild parr (---) and wild downstream migrants (B). Expected pectoral fin lengths were estimated from the logarithm of the equations in Table 3. Three-point averaging was used for smoothed curve estimates of A, B.

Rocky Brook population (Table 2). Heritabilities estimated within each population were less than 5% ($h^2 \times 100$), in all but two comparisons. Heritabilities estimated for each trait when all families were pooled were consistently less than 3%. The similarity of the variance components for all traits analyzed clearly results from their strong genetic correlations (Table 5).

While the relative importance of the variance components must be interpreted with some caution, the relatively small contribution of additive variance to the total phenetic variance and the consistency of the estimates of its magnitude between studies (Refstie and Steine 1978; Refstie et al. 1977) suggests that additive variance for growth and body morphology is low within natural populations. Since the adaptive value of traits is generally believed to be inversely proportional to the magnitude of the additive variance (Fisher 1930), selection for growth rate and body form has probably been strong in Atlantic salmon.

In animal improvement programs, estimates of additive genetic variance are commonly applied in the prediction of selection gain. In an ecological context, however, the role of additive genetic variance in the maintenance of fitness in natural populations which experience variable environments is of primary interest. Such an understanding is also important to the development and assessment of management strategies since many current practices can potentially influence the genetic structure of populations. Additive variance related to population fitness should decrease in response to selection (Fisher 1930). Investigations have consequently attempted to explain how selection could maintain the apparently high levels of genetic heterogeneity (reviewed in Hedrick et al. 1976; Lewontin 1974; Weins 1976). These investigations have led to a number of hypotheses concerning the adaptive value of maintaining additive genetic variance. These include the increased probability of population survival through time; compensation for short-term environmental variation, or the tracking of longer-term environmental fluctuations. The maintenance of additive variance in Atlantic salmon populations will be assisted by the spatial separation of spawning populations (Levene 1953; Levins 1968; Christiansen 1974; Strobeck 1974). Dominance effects, strong genetic correlations, and spatial or temporal variability in selection intensities will also contribute to its maintenance (Levins 1965; Emlen 1973; Karlin and Levikson 1974; Hedrick 1976). Levins (1965, 1968) hypothesized that an intermediate level of additive variance should exist in populations occupying heterogeneous environments since excessive additive variance increases segregation load. Levins proposed that an optimal genetic system should possess additive variance on the order of 2−10% of the environmental variance. The additive variance we observed in these natural populations (3−5% on average) is within this range for the predicted optima in selected traits.

Directional selection was hypothesized to account for the significant interpopulation differences in allometric exponents for pectoral and pelvic fin length. The departure of the pectoral fin length of fish collected in the natural environments from the expected fin length estimated from the allometric relationship calculated from fish reared in the hatchery was used to evaluate this hypothesis. An underlying assumption of this evaluation was that the hatchery environment did not significantly alter the allometric relationship. The higher temperatures and rearing densities in the hatchery could affect them. Rearing densities were equal between families used in the estimation of the allometric regressions and were generally very low by hatchery standards (100 fish per tank). No evidence of any fin rot or abrasion was observed. With respect to the temperature differences, Martin (1949) demonstrated a positive relationship between relative pectoral fin length and temperature for rainbow trout. Any effects due to elevated temperatures in the hatchery would thereby strengthen our test.

Frequency distributions of the observed pectoral fin length of wild fish minus the expected pectoral fin length divided by fork length had marked positive deviations from the expected values (Fig. 5). Selection intensities (I) and percentage increase in fitness ($\%w$) were calculated by the method of O'Donald (1970; Rocky Brook I = 0.21, $\%w$ = 4.5; Sabbies River I = 0.13, $\%w$ = 1.8). Selection intensity was 1.6 times greater in the Rocky Brook population, resulting in a 2.7% increase in mean fitness per generation. Evidence of genetic variation for body morphology, low additive genetic variance, and strong directional selection for larger fin size in Rocky Brook, the habitat which experiences a 1.25 times greater average flow velocity, supports our hypothesis that larger relative fin size is adaptive in environments with greater

flow velocity. Genetic variation is expressed very early in development as exemplified by the constancy of the between-population ratio for relative fin size at swim-up (Rocky Brook : Sabbies River = 1.1:1.0) and at 10-cm mean length (1.12:1.0).

In sharp contrast to the differences noted in body morphology between the Rocky Brook and Sabbies River populations, growth rate and proximate composition were very similar. Daily growth increments and proximate compostion of parr were also similar in the natural environment (Riddell and Leggett 1981). The higher additive variance relative to the dominance components for fat-free dry matter (FFDM, Table 2B) suggests that the genetic contribution to this homeostasis for body compostion has an additive genetic basis. A strong contribution of additive variance to genetic homeostasis is predicted when temporal variation exceeds spatial variation (Bryant 1976; Levins 1968, Valentine 1976) as in the case with the pronounced seasonality of temperature in northern latitudes.

The demonstration of adaptive genetic variation between local populations of *Salmo salar* (Atlantic salmon), coupled with similar findings by Brannon (1967), Raleigh (1971), and Bowler (1975) for other salmons and trout, strongly supports an implied genetic basis to phenotypic variation in the Salmonidae (Ricker 1972; Schaffer and Elson 1975; Gardner 1976). The significance of genetic variation to the management of salmonid populations has recently been emphasized in restoration and enhancement programs (Loftus 1976; Larkin 1979). Such management practices should seek to protect the genetic integrity of locally adapted populations if fitness is to be maintained and reservoirs of genetic variation made available for future management applications.

Although the potential detrimental effects of reduced population size (Ayala 1968; Ihssen 1976) and unnaturally high gene flow created by hatchery supplementations (Bams 1976; Utter et al. 1976; Reisenbichler and McIntyre 1977; Krueger and Menzel 1979) are now recognized, there remains a distinct lack of knowledge concerning the genetic regulation of phenotypic variation. Electrophoretic studies of protein variation correlated with phenotypic traits and the additive genetic variation within populations may have considerable potential for expanding this understanding (Allendorf and Utter 1979). It is also noteworthy that interpopulation differences accounted for 20% of the phenotypic variation in body morphology in the natural Rocky Brook and Sabbies River populations (Riddell and Leggett 1981). This value was very similar to the portion of the phenotypic variance accounted for by the combined genetic components in this study. Discriminant function analysis applied to quantitative traits, as in Riddell and Leggett (1981), may also prove to be a useful technique for estimating the genetic divergence between populations (see also discussions by Rao 1953; Sokal 1961; Goodman 1969).

In view of the extensive electrophoretic and phenotypic variability exhibited by the Salmonidae, it is highly probable that as knowledge of the adaptive basis of this variability expands, it will rapidly become impractical to manage each recognizable genetic unit. One possible solution to this problem may be to direct future studies of the adaptive basis of genetic divergence towards the definition of functional management units based on stepped clines (Endler 1977) and/or adaptive life history parameters (Saunders 1967; Schaffer and Elson 1975; Leggett and Carscadden 1978). The potential advantage of delineating management units based on stepped clines derives from the fact that selection differentials must change rapidly over a limited distance and gene flow must be low across the clinal boundary for these clines to persist. Populations contained within a management region defined by such clines should, therefore, be more similar genetically than populations located beyond clinal boundaries. Defining management units as localized groups of genetically similar populations, delineated on the basis of a hierarchy of adaptive traits, provides a compromise between managing each genetically variable population and the available management effort while minimizing potential genetic effects due to man's influence.

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A Model for Responses of Vertebral Numbers in Fish to Environmental Influences During Development

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LINDSEY, C. C., AND A. N. ARNASON. 1981. A model for responses of vertebral numbers in fish to environmental influences during development. Can. J. Fish. Aquat. Sci. 38: 334–347.

A model is proposed in which the number of vertebrae formed in an embryo is determined by two independent processes each building up with time. The processes may possibly but not necessarily represent growth and differentiation. Final number is fixed by the level that one process has attained when it is suddenly terminated by arrival of the second process at a critical level. Varying the temperature or other environmental conditions during development can differentially affect the time courses of the two processes and hence alter the numerical outcome. The model, termed "atroposic," is shown to encompass a welter of apparently conflicting published data on vertebral responses of many fish species to different constant temperatures or to single or double temperature changes. It suggests how vertebral numbers may respond strongly and in either direction to temperature perturbations, and how the responses may be extralimitary, and how large responses may be produced by quite brief temperature pulses. Concepts of several temperature-sensitive periods and of shock effects are abandoned. The model is shown to be capable of computerized fit to almost all published data on variation in vertebrae, but to only some on variation in fin-ray number. The atroposic model seems to approximate reality, but the present mathematical form may be only a special case of a more general form yet to be developed.

Key words: environmental conditions, meristics, models, temperature responses, vertebrae counts, *Salmo trutta, Oryzias latipes, Plecoglossus altivelis*

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Les auteurs proposent un modèle dans lequel le nombre des vertèbres formées dans un embryon est déterminé par deux processus indépendants, chacun se développant en fonction du temps. Ces processus peuvent, bien que non nécessairement, représenter la croissance et la différenciation. Le nombre final est fixé par le niveau atteint par un processus au moment où il se termine brusquement par l'arrivée du second processus à un niveau critique. Des variations de température ou d'autres conditions ambiantes durant le développement peuvent influencer différenciellement le cours des deux processus et, partant, altérer les nombres. On démontre que le modèle, appelé « atroposique », englobe un fouillis de données publiées, apparamment contradictoires, sur la façon dont les nombres de vertèbres chez plusieurs espèces de poissons réagissent à différentes températures constantes ou à des changements simples ou doubles de température. Le modèle donne une idée de la façon dont les nombres vertébraux peuvent réagir fortement et dans l'une ou l'autre direction à des perturbations thermiques, comment les réponses peuvent être extralimitaires et comment des pulsations thermiques assez brèves peuvent produire de fortes réponses. On abandonne les concepts de plusieurs périodes de sensibilité à la température, ainsi que ceux des effets de chocs. Le modèle se montre capable d'application, sur ordinateur, à presque toutes les données publiées sur la variation du nombre des vertèbres, mais à seulement quelques-unes sur la variation du nombre des rayons des nageoires. Le modèle atroposique semble se rapprocher de la réalité.

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Il se peut toutefois que la présente forme mathématique ne soit qu'un cas particulier d'une forme plus générale, encore à découvrir.

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WE propose here a model offering coherence to the scattered published information on how vertebral numbers in fish respond to environmental influences during early development. No unifying concept has been offered previously for the welter of conflicting experimental data. In this paper meristic responses to the environment are discussed only at the level of the individual embryo, but there are also implications at the level of fisheries management, since identification of fish stocks often depends heavily on meristic characters such as vertebrae, fin rays, or scale rows.

Rearing embryos at different sustained levels of temperature, light, or oxygen produces a variety of meristic responses. There are different responses to the same factor in different species, and to different factors in the same species. Even greater disparity emerges when an embryo is moved between two environmental levels during development. Sometimes the resulting meristic count is higher than counts produced by either sustained level; sometimes it is lower, and sometimes both extralimitary responses can occur depending on the developmental stage at which the move occurs. Brief pulses of high or low temperature applied at various stages may also lead to striking meristic responses. These apparently inconsistent data have resisted satisfactory synthesis. Experiments have mainly involved fish, but also amphibians, reptiles, birds, and mammals.

Our first attempt to cut this Gordian knot was inspired by a paper by Carl Hubbs (1926). He postulated that temperature controls the number of parts produced in embryos by differentially affecting the rates of growth and of differentiation: at low temperatures, growth is inhibited less than is differentiation, the embryo becomes longer before it segments, and therefore more parts are formed. Several observations on wild fish (Gabriel 1944) are compatible with the hypothesis, including the high scale counts of fish in which parasite infection had caused delay of scale formation until the fish were longer than usual. Hubbs suggested no quantification, and few laboratory experiments on the question were available at the time.

We apply here a quantified version of Hubbs' concept to published experimental studies on vertebral variation. The resulting mathematical relationships provide a consistent framework for previously unintegrated results. The mathematical interactions seem, therefore, to approximate reality. But the embryological processes which may correspond to these mathematical components rest as yet on no observational base. In fact we suspect that "growth" and "differentiation" may not be the most appropriate terms for the processes involved. Even so, the success of the mathematical model in fitting experimental data can be judged, independent of conjecture as to what real processes the mathematics may represent. What is demonstrated here is that vertebral numbers respond to developmental environments as though controlled by processes which interact mathematically in the way the model suggests. The model's principal value may be to provide for the first time a quantitative hypothesis susceptible to disproof. Future rearing experiments or embryological measurements may lead to rejection or to modification of the model or they may provide clues to the actual developmental mechanisms represented by the equations.

Description of the Atroposic Model

In generalized terms, the model supposes that a quantitative outcome (here the final number of vertebrae formed) results from two independent processes each building up with time. The outcome is fixed by the level that one process has attained when it is suddenly terminated; termination is triggered at the moment when the other process has risen to some critical level. Varying the conditions may differentially affect the time courses of the two processes and hence alter the outcome.

To stress the general nature of the model while preserving the anonymity of its components, we adopt a classical symbolism. The three Fates of Greek mythology provide a convenient metaphor. One, Clotho, was said to spin the thread of life. A second, Lachesis, drew off the thread and controlled its course. The third, Atropos, then cut off the thread, rendering the outcome of her sisters' work irreversible. The class of model described above can be named "atroposic," because of its feature of termination at a critical moment. That process which controls the moment of termination can be labeled as Atropos. The other, whose level measures off the outcome, is Lachesis. If parallels were sought with Hubbs' concept, L might be thought of as representing length of elongating tissue (i.e. "growth"), and A as allocation (i.e. "differentiation"). Clotho's role is currently unquantified.

The atroposic model can be described more precisely in two forms — either graphical or mathematical. The graphical version of the model will be stressed in the body of this paper. It can be followed without recourse to (or indeed without specifying) the mathematical basis of the curves. The mathematical version, and procedures for model fitting and computation, will be outlined in the appendices.

The heavy lines on Fig. 1 show the time curves of the L-process and A-process, in an embryo reared either at sustained high or low temperature. At high temperature, A rises steeply and reaches the threshold level c at the time t_{hi}; the resulting number of vertebrae is determined by the level V_{hi} to which L has risen at time t_{hi}. At low temperature A reaches the threshold later, at time t_{lo}, by which time L has risen to the level V_{lo}. In Fig. 1 the curves are so arranged that fewer vertebrae are formed at high than at low temperature, but slightly different slopes can produce fewest vertebrae at low temperature, or at an intermediate temperature. Indeed, so long as the model is applied only to effects of sustained temperatures, it is so flexible as to have little predictive value.

The value of the atroposic model emerges when it is applied to effects of temperature changes during development. The

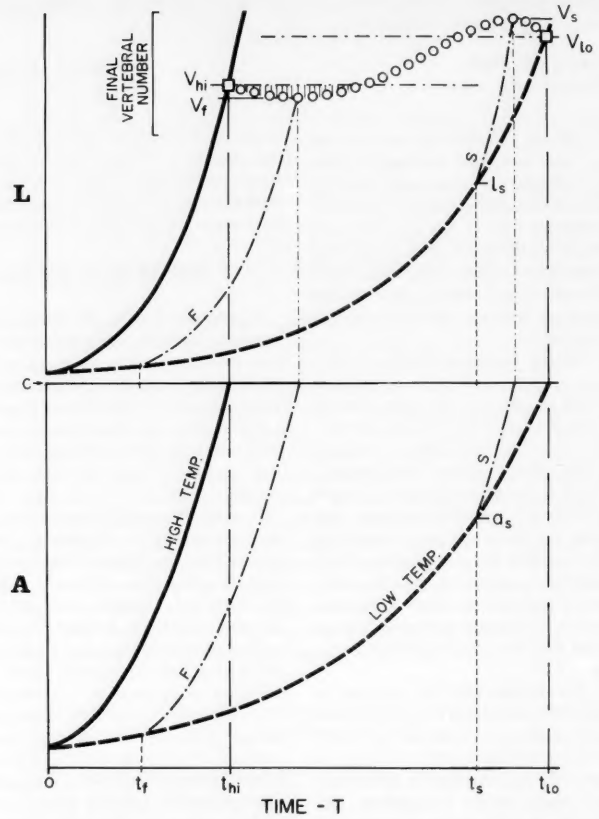

FIG. 1. Hypothetical "atroposic" model for determination of vertebral number. Development of *L*-process and of *A*-process follows heavy solid curves at constant high temperature, heavy broken curves at constant low temperature. *F* and *S* are portions of developmental curves followed after transfer from low to high temperature at times t_f or t_s, respectively. Resulting vertebral numbers shown by squares (constant temperature) or circles (transfers from low to high temperature). For further explanation see text.

outcome of shifting from low to high temperature at either of two developmental times can be traced on Fig. 1. In the first example, both *L*- and *A*-processes proceed slowly following their respective low temperature curves, until the embryo is shifted to high temperature at time t_f. Each process then proceeds faster, parallel to its high temperature curve, along the segments labeled *F*. The final vertebral number is determined by the height, V_f, reached by the *L* curve at the moment when the *A* curve reaches the threshold *c*. In the second example, a later shift from low to high temperature, at time t_s, causes *L* and *A* to follow the segments labeled *S* after transfer, and produce a final number V_s. For the particular curves shown here, a series of shifts from low to high temperature at intermediate times will produce counts shown as open circles in Fig. 1. Early transfers produce counts below those arising from either sustained temperature, but late transfers produce counts above.

Temperature shifts in the opposite direction (not shown here) can be simulated in the same way, with *L* and *A* starting along the high temperature curves until transfer, and then paralleling the low temperature curves. The resulting counts show a pattern reversed from those in Fig. 1; early transfers produce counts above the sustained temperature values, but late transfers produce counts below. More complex temperature changes involving two or more shifts can also be simulated.

Terminology

"Atroposic" will be used as referring to the class of model described above, in which one process, by reaching a critical value, terminates the progress of a second process. The development of the first process is designated as the "*A*-curve" (Atropos, perhaps allocation or differentiation), the second as

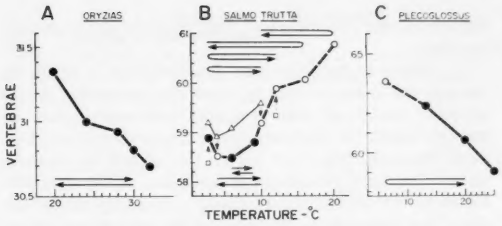

FIG. 2. Experimental results of rearing three fish species at constant temperatures. Solid circles are mean vertebral counts from one set of parents for each species. In B, open circles are estimates, based on means from other sets of parents (shown by triangles or squares) which were adjusted by amount indicated by small vertical arrows as explained in text. Horizontal arrows indicate the single or double temperature transfers on which the experimental results in Fig. 4 and 5 are based.

the "*L*-curve" (Lachesis, perhaps length or growth or, as suggested later, cell number). An atroposic model might be fitted to any meristic series, not just vertebrae, and might examine the effect of any environmental variable, not just temperature. The term is not restricted to any particular mathematical form.

Some other terms, none new, are defined here because their previous usage has been ambiguous or inconsistent. In description of the response of meristic counts to an environmental variable such as temperature, the curve is "acclivous" (sloping up) if the counts are progressively higher at higher temperatures. The response is "declivous" (sloping down) if the counts are progressively lower at higher temperatures. Curves are "V-shaped" if the minimum count is at an intermediate temperature, or "arched" if the maximum count is at an intermediate temperature. (The term "count" hereafter refers to the mean number of countable parts in a sample of fish, and does not imply a whole number).

A temperature "break" experiment is one in which developing embryos are abruptly transferred from one temperature to another and retained at the new temperature until the meristic count is fixed. A temperature "pulse" experiment is one in which all development occurs at one ("control") temperature, except that the embryos are abruptly transferred to a new temperature for a specified period and then returned to the control temperature for the duration of their development. Break or pulse experiments can be conducted on environmental variables other than temperature, such as light intensity.

The meristic response to a temperature break or pulse is said to be "extralimitary" if the resulting count lies outside of those counts which would have arisen from sustained rearing at either the low or the high temperature. The extralimitary response is an "overcompensation" if it lies in the direction of but beyond the count for sustained rearing at the temperature to which the embryos had been permanently moved (in a break), or temporarily moved (in a pulse). The extralimitary response is "paradoxical" if it lies in the direction of but beyond the count for sustained rearing at the temperature from which the embryos had been moved (i.e. if the response is in the direction opposite to what might be expected). The classification of a particular extralimitary response as overcompensation or paradox therefore depends not on whether the response was a rise or a fall, but instead on the direction of change relative to the results of sustained rearing. In Fig. 1 the vertebral number produced by sustained low temperature, V_{lo}, lies above the number produced by sustained high temperature, V_{hi}. In the first temperature break shown, transfer from low to high temperature at time t_f produced a vertebral number V_f which is below V_{hi}, hence an overcompensation; in the second break shown, transfer in the same direction but at time t_s produced a vertebral number V_s which is above V_{lo}, hence a paradoxical reaction. The responses to all early breaks within the hatched area in Fig. 1 are overcompensations; responses to late breaks, within the stippled area, are paradoxical reactions.

Materials and Methods

SOURCES OF DATA

To illustrate how the atroposic model can be fitted to experimental results previously published but not previously integrated, three of the several fish species examined will be considered in detail. For each species, vertebral counts are available from rearing at two or more sustained temperatures, and also from one-time temperature transfers ("breaks"), or from temperature changes of short duration ("pulses"), or from both. The array of published experimental data is summarized in Fig. 2.

Data available for model fitting differ among the three species in nature and reliability. The most extensive experiments have been on the brown trout, *Salmo trutta*, for which there are vertebral counts from sustained rearing at many temperatures, four break experiments (each at a single time), and four pulse experiments (at one to several times). Means are based on large sample sizes (usually 100) and have small confidence intervals compared with the other two species. Results have been integrated from Danish studies on the migratory form by Tåning (1944, 1950, 1952) (which he refers to as "sea trout" *Salmo trutta trutta*), and from Polish studies on the nonanadromous form by Orska 1956 (which she refers to as "brook trout" *Salmo trutta* morpha *fario*). To compensate for differences between absolute vertebral counts produced by embryos reared at the same temperature but arising from different parents, one set of parents (H × G of Tåning 1944) was selected as standard. Mean counts of embryos from all other parents were adjusted upward or downward by an absolute amount chosen so that vertebral response curves to sustained rearing temperatures were approximately superimposed. This procedure assumes that response curves of different genotypes are similar in their shape although different in absolute vertebral number. Justification for the procedure is provided by Fig. 2 of Tåning 1952, which shows that, in plots of vertebral means against rearing temperature for 10 sets of brown trout parents, all curves approximate a *V*-shape with apices roughly coinciding although with large displacements along the vertebrae axis.

Sources of vertebral counts for *S. trutta*, and adjustments, were Tåning 1944, p. 23, parents H × G (Fig. 2B solid circles, Fig. 4E, F); Tåning 1950, p. 28, Tåning 1952, p. 175,

means adjusted upward by 0.54 vertebrae to conform to parents H × G (Fig. 2B squares, Fig. 5A, B); Tåning 1952, p. 183, means adjusted downward by 0.43 vertebrae (Fig. 2B triangles, Fig. 4C, D); Orska 1956, p. 313–315, parents I adjusted upward by 0.19 vertebrae, parents II downward by 0.17, parents III downward by 0.85, parents IV downward by 0.10 (Fig. 5C, D). Means for two highest temperatures in Fig. 2B were estimated by extrapolation.

Vertebral counts for medaka, *Oryzias latipes*, a cyprinodont fish, are available for five sustained temperatures and two series of temperature break experiments (Fig. 2A, 4A, B). The break series are more extensive and more evenly spaced in time for this than for other species. Only offspring from a single set of parents were used (Ali and Lindsey 1974, unpublished data, parents U), so no adjustment for different genotypes was needed.

For the ayu, *Plecoglossus altivelis*, a salmoniform fish, vertebral counts are available for only three sustained temperatures and one series of temperature pulse experiments, based on a single batch of eggs with mixed parentage (Fig. 2C, 5E). The mean vertebral count for 6°C was estimated by extrapolation both from hatchery- and wild-reared fish (Komada 1977a). This is the least extensive set of data, but is presented because it includes a very early temperature pulse resulting in a surprising vertebral response.

In fitting the model, it is necessary to know not only the vertebral counts but also the "completion times," i.e. the times at which the A-process reaches the threshold c. Where data were available from a series of temperature break experiments, completion time was estimated (for the temperature which preceded the break) as that time after which vertebral count could no longer be influenced. Once the embryonic stage corresponding to completion time was established for one temperature, then completion times could be estimated from published tables of developmental times at other temperatures. Choice of completion time was the best compromise between results of all break or pulse data. In all cases the temperature coefficients of the designated completion times were similar to those known for times to reach some other developmental stages.

For *S. trutta*, durations of several embryonic stages at different temperatures, and hence temperature coefficients, were available from Garside (1966b), Hallam (1974), and elsewhere. Completion time for vertebral number corresponded to stage 11 of Hallam (initiation of extraembryonic vascularization). At extreme temperatures, for which no published data on developmental times were available, best fits were obtained if completion times were slightly longer below 3°C and slightly shorter at 16 and 20°C than would be predicted from a straight line log–log plot of completion time against temperature.

For *O. latipes*, temperature coefficients for several stages were available from Ali and Lindsey (1974), and Ogawa (1971). Completion time for vertebrae corresponded to stage 26 of Ali and Lindsey (1974), that is, roughly equivalent to the same stage in *Fundulus heteroclitus* of Oppenheimer (1937), or about to stage 29.5 of Ogawa (1971).

For *P. altivelis*, temperature coefficients were available only for time to hatching (Komada 1977b). The vertebral completion times shown in Table 1 have the same temperature coefficients, but these have been approximated from very few data.

In addition to the three cases described here in detail, an attempt was made to analyze, from the perspective of the atroposic model, all other available data on responses of meristic counts to environmental changes during development. Scattered data exist for 12 species and six meristic series. The same procedures as described above were followed for assembling meristic counts, estimating completion times, and fitting the model. Some of the cases examined are described briefly in the Results.

Procedures

We calculated curves for the A- and L-process that provided the best fit to the experimental data. At each temperature, the equations for the two processes shown in Fig. 1 are of the exponential form (for reasons given in Appendix A):

$$A = \alpha_1 (\exp[\alpha_2 t] - 1)$$
$$L = \lambda_1 (\exp[\lambda_2 t] - 1)$$

where t is time in days since fertilization, α_1 and α_2 are the two parameters governing the shape of the A-curve, and λ_1 and λ_2 are the two parameters governing the L-curve.

Inputs to calculating these equations for each species were the vertebral means resulting from rearing at various sustained and shifted temperatures, and also the completion times at sustained temperatures. Sources of these data have been given above. Outputs from the calculations were, for each temperature, the parameters α_1, α_2, λ_1, and λ_2. (Two of these, α_2 and λ_2, are constrained once the other variables are fixed.) The methods of mathematical calculation are described in Appendices B and C. Resulting parameters are given in Table 1.

The sequence followed in calculating equations for any two temperatures was to impose the restraints that each temperature if sustained must produce precisely those vertebral counts actually observed; within these restraints the curve shapes were then chosen by computer optimization so as to best approximate the results of break or pulse experiments. To illustrate, each of the heavy A-curves and L-curves in Fig. 1 may be thought of as fixed at two points, one at the origin and the other at the intersection between the time to completion and either c or the final vertebral number. But even with these constraints there could be different curve shapes which would produce different vertebral numbers in embryos that were transferred between the two temperatures during development. The best shapes to fit any set of experimental transfer results can be arrived at by iterative trials (which will reveal the best combination of the parameters α_1, α_2, λ_1 and λ_2).

The fitting program can use data from breaks and/or pulse experiments in either or both directions between two temperatures. Where all available data are used to fit the model, adequacy of fit merely demonstrates that the model is sufficiently flexible to give consistently good fits for the experimental results. If the model involves relatively few parameters and the variety of experimental results is great, this makes the model more credible. Much stronger evidence of model validity is obtained if the results are stratified into

TABLE 1. Parameters of the exponential L- and A-curves in Fig. 3, vertebral means, and completion times, for rearing at sustained temperatures.

Species	Temp, °C	Mean no. vert.	Compl. time, days	L-curve		A-curve	
				λ_1	λ_2	α_1	α_2
Oryzias latipes	20	31.3	10.4	0.4244	0.4148	4.7902	0.1084
	30	30.8	4.7	0.5092	0.8763	6.0020	0.2086
Salmo trutta	2.5	58.9	62	7.8	0.03470	0.00379	0.1271
	4	58.5	40	9.9	0.04832	0.00550	0.1877
	6	58.5	29	12.3	0.06034	0.00750	0.2481
	9	58.8	19.5	15.2	0.08117	0.01050	0.3518
	10	59.2	17.5	16.1	0.08818	0.01132	0.3877
	12	59.9	15.5	19.0	0.09190	0.01240	0.4319
	16	60.1	11.5	24.6	0.1075	0.01430	0.5697
	20	60.8	9	30.0	0.1231	0.01600	0.7155
Plecoglossus altivelis	6	63.6	31	23.74	0.0420	0.01350	0.2133
	20	60.6	7.7	15.01	0.2100	0.00790	0.9271

two sets, with only the first set being used for the fit. The fitted model is then used to predict the results in the second set. This could be done in some of the *S. trutta* data, where the pulse experiments alone gave good model fits (as evidenced by small standard errors for the estimated parameters and quick convergence of the iterative fitting procedure). All parameters in the range 2.5−10°C were calculated from Orska's pulse experiment (Fig. 5C). The results of six of Tåning's break experiments involving temperatures within this range were then predicted and found to all lie within four standard errors of the observed means (four of the experiments shown in Fig. 4C, D, E, F). This prediction is more than just a simple interpolation of the model. The type of experimental results in the set to be predicted differs in kind from the type used for fitting, so the prediction is a model extrapolation that depends critically on the model structure.

When three or more temperatures were involved in experimental data, a satisfactory family of parameters for all temperatures was arrived at by compromise between the parameters calculated from temperature pairs. Each of the four parameters at eight temperatures for *S. trutta* (Table 1) falls along an approximately straight line (slightly inflected above 10°C) when plotted against temperature on log−log axes.

Results

The calculated L- and A-curves are shown in Fig. 3 for three species. Table 1 lists their parameters. Figure 4 allows comparison, for two of the species, between actual vertebral means produced by temperature break experiments and the pattern of vertebral responses (shown as heavy lines) which would be expected if vertebral number were determined according to the atroposic model in Fig. 1 using the curves in Fig. 3. A similar comparison between observed and theoretical responses is provided in Fig. 5 for temperature pulse experiments.

Table 1 gives parameters only for those temperatures which were used in the break or pulse experiments (summarized in Fig. 2). Curve equations for any intermediate temperature can be calculated by interpolation between parameters in Table 1 (log−log plot) and will be found to fit the vertebral means in Fig. 2 observed from sustained rearing at that temperature.

The Model Fitted to Temperature Break Data

Vertebral counts of *O. latipes* respond to rearing temperatures much as in the hypothetical example in Fig. 1. Sustained rearing at low temperature (20°C) produces high counts, and at high temperature (30°C) produces low counts (Fig. 2A). Temperature breaks from 20 to 30°C produce significantly low extralimitary counts (overcompensation) if applied at 2 d after fertilization, or significantly high extralimitary counts (paradox) if applied at from 6 to 10 d (Fig. 4A). Temperature breaks in the opposite direction, from 30 to 20°C, were tried at only 4 times, the earliest of which produced significantly high counts (again, overcompensation).

The fitted model (heavy lines in Fig. 4A, B) using parameters in Table 1 achieves a good approximation of all these observed data. Temperature breaks in either direction produce overcompensation if the break is early, paradox if it is late. Of these, only the late paradox in Fig. 4B is not evident in the data, and even here the fitted line is included within the 95% confidence intervals (as it is for all but one of the 15 experimental means).

For *S. trutta*, temperature break data are sparse. In each of two breaks from low to high temperature (Fig. 4C, E), and in the one from high to low (Fig. 4D), the experiment was applied late in development and the result was a significant paradox. The fourth break shown (Fig. 4F), and others described by Tåning (1944), were probably applied after vertebral count had become fixed, and produced no extralimitary responses. The fitted model succeeds in encompassing all these data, as well as the more complex results of pulse experiments described below (Fig. 5 A, B, C, D); the late paradoxes conform to the observations, but no break experiments have been performed to check the predicted early overcompensations.

As well as the two fish species for which break experiments have been analyzed in detail above, several others were used to test the applicability of the atroposic model. Results are summarized below.

Vertebral counts of rainbow trout, *Salmo gairdneri*, pro-

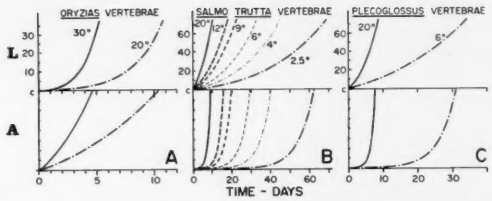

FIG. 3. Curves of *L*-process and *A*-process at different temperatures for three species, calculated following the atroposic model in Fig. 1 so as to give the vertebral numbers produced experimentally by constant rearing temperatures (Fig. 2), single temperature transfer breaks (Fig. 4), or temperature pulses (Fig. 5). Parameters given in Table 1. All temperatures in this and following figures are celsius.

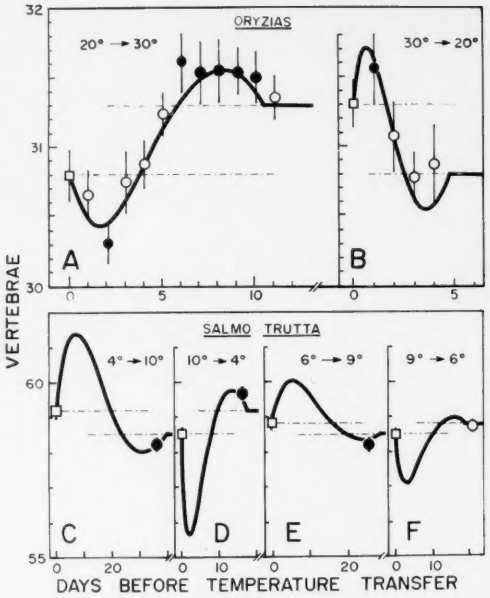

FIG. 4. Vertebral counts in two species resulting from temperature breaks (single transfers) between the indicated temperatures, at times following fertilization shown by horizontal axis. Heavy lines show vertebral counts which would result according to the atroposic curves given in Fig. 3 and Table 1; circles and squares are actual data (in some cases adjusted for parent differences as explained in Materials and Methods). Solid symbols are significantly different ($P < 0.05$) from both control values, which are shown as squares and horizontal broken lines. Vertical bars are 95% confidence intervals (scarcely visible beyond symbols for some means).

duced by constant temperatures and by temperature breaks were available from Hallam (1974) and from our unpublished experimental data. Several authors have found that in rainbow trout vertebral response to rearing temperature is declivous. Hallam's data on rainbow trout and on brook trout are among the best for any species, including temperature breaks from 9 to 12°C at 6 times, from 12 to 9°C at 7 times, and from 6 to 9°C at 4 times, all on eggs from the same parents. Each series produced overcompensations if the breaks were early, and paradoxes if the breaks were late. We calculated parameters for 6, 9, and 12°C which allowed a good model fit to all these data. A single aberrant point, paradoxical reaction in the earliest transfer from 12 to 9°C, may as suggested by Hallam have been due to an experimental mishap (although suggestions of a similar response appear in our own unpublished data).

Our own experiments, using an inbred hatchery stock of fall-spawning *S. gairdneri*, tested breaks between 4 and 12°C in both directions, and two temperature pulses. Again, breaks produced overcompensations if applied early, or paradoxes if applied late. A single family of parameters would not encompass both Hallam's and our results, because of large genetic differences between the two stocks in the degrees of vertebral response to constant rearing temperature. Nevertheless, the model could be fitted, using slightly different parameters, to either set of experiments, and the general patterns of response to temperature breaks were similar in both.

Vertebral counts of brook trout, *Salvelinus fontinalis*, are available from Hallam (1974) and cover experiments similar to those he conducted on rainbow trout. Response to constant rearing temperatures was declivous. Breaks in both directions between 9 and 12°C, and from 6 to 9°C, all produced overcompensation if early, paradox if late. An internally consistent family of parameters was calculated which produced a good model fit to all these data.

The self-fertilizing cyprinodont *Rivulus marmoratus* showed strong vertebral overcompensation in early temperature breaks in either direction between 25 and 30°C (Swain 1979). Previously, early temperature breaks from 26 to 20°C had been found to show a suggestion of overcompensation (Lindsey and Harrington 1972, Fig. 1B). Vertebral response to temperature is declivous (Harrington and Crossman 1976). The data can all be fitted by the model, although interpretation is complicated by a "prefertilization effect." This phenomenon, in which parental temperature before fertilization affects offspring vertebral count, was suspected in *Brachydanio rerio* (Dentry and Lindsey 1978) and has now been substantiated in *R. marmoratus* (Swain 1979). The latter species has the advantage in meristic experiments that individuals are genetically uniform, which removes the possibility of selective mortality as a source of diversity among survivors (Lindsey and Harrington 1972).

Vertebral counts of the cyprinodont *Fundulus majalis* have been reported from rearing at each of several mean temperatures (the temperature actually fluctuating 2°C above and below the mean each day) (Fahy 1972, 1976). Response of vertebrae to sustained temperatures was declivous. Responses were also recorded for breaks to high from low or medium temperatures, and for breaks to low from medium temperatures. Late breaks to high from medium temperatures produced paradoxical response in vertebrae. One family of parameters was found that allowed the model to fit all these data.

In addition to the experiments on medaka, *O. latipes*, reported in detail above, two other publications deal with this species. Ogawa (1971) used a mutant strain "white fused

fbr'' to study effects of temperature breaks on vertebral count. His data, like those of Ali and Lindsey (1974), showed an overcompensation in an early break from 20 to 28°C, but no other extralimitary responses.

Lindsey and Ali (1965), using a different set of medaka parents from those in the experiment analyzed here, tested the effect of alternating the temperature between 20 and 30°C every 12 h throughout development. This may be thought of as a series of temperature breaks in opposite directions. Despite the fact that the embryos were subject to the shock of sudden temperature change twice a day, the final vertebral count lay right between the counts from sustained rearing at either high or low temperature. Although we have not carried out the complicated mathematical analysis, it is evident from graphical analysis that our atroposic model would predict just the sort of intermediate count that was observed.

The Model Fitted to Temperature Pulse Data

Four sets of temperature pulse experiments for *S. trutta* are shown in Fig. 5 A, B, C, D: two in which a low temperature pulse was applied for 8 or 8.8 d, and two in which a high temperature pulse was applied for 1 or 1.25 d. The pulses all lasted for roughly comparable numbers of thermal units or day-degrees (duration multiplied by degrees above biological zero, a convenient although somewhat misleading measure of embryonic progress). One series (Fig. 5C) included pulses at several times; the earliest pulse produced a significant vertebral overcompensation, and two of the later pulses produced significant paradox. The other three series, which tested the effects of late pulses only, each produced a significant paradox. All these pulse results, as well as all the results of temperature breaks, can be seen from the heavy lines in Fig. 4 and 5 to be fitted by the model, using the family of parameters in Table 1. Initial fitting was performed on the pulse data, which were more numerous than the break data. Predictions of the outcome of breaks, using parameters calculated from pulses, were correct in general pattern; the parameters used here are a compromise which provided the closest fit to all the sets of experimental results.

A series of short low temperature pulses was applied to *Plecoglossus* embryos which were otherwise held at high temperature. Vertebral count which would result from rearing continuously at the pulse temperature 6°C was estimated by extrapolation (Fig. 2C) to be so high that it lies off the top of Fig. 5E. A 1-d pulse starting just 1 h after fertilization produced a significant paradox in vertebral count. Such response to very early pulses is also predicted for *S. trutta* (Fig. 5A, B, C), but only in *Plecoglossus* has an early pulse actually been tried. Pulses at 2 or 4 d produced counts significantly above the control (although not extralimitary), but the pulse at 3 d did not. Although the experimental data for *Plecoglossus* are sparse and the confidence intervals wide, it can be seen from the heavy line in Fig. 5E that the model has approximated both types of significant departure from the control.

The only available vertebral responses to temperature breaks or pulses to which we have been unable to fit the atroposic model are those of Orska (1956, 1962) for rainbow trout, *S. gairdneri* (referred to by her as *Salmo irideus*). Her series of pulse experiments each produced two or three apparent abrupt shifts between overcompensation and paradox, but without consistent pattern. To provide adequate sample sizes in the face of heavy mortalities, she used three or four sets of parents to make up each series of pulse experiments. Some of the apparent sudden reversals in direction of vertebral response may have been due to genetic differences between the parents of successive samples within the one series. These studies were carried out in the face of great technical difficulties including heavy water chlorination and imprecise temperature control. Temperature breaks, on the same species in North America, described earlier, do not display comparable reversals. Whether rainbow trout actually show such complex vertebral responses to temperature pulses will be resolved only by experiments with lower mortalities and adequate genetic control.

Environmental Factors Other than Temperature

Vertebral responses to transfers between different light levels are suggestive of vertebral responses to temperature transfers, although the evidence is fragmentary. Sockeye salmon, *Oncorhynchus nerka*, were subjected to ''light breaks'' (i.e. were transferred between two different regimes of light intensity and duration) at various developmental stages (Canagaratnam 1959). Paradoxical responses of vertebrae occurred in some lots, overcompensations in others. Chinook salmon, *Oncorhynchus tshawytscha*, were reared in near darkness except for a 5-d pulse of light, which varied between experimental lots in its intensity and developmental stage of application (Eisler 1961). Early pulses of bright light (at days 1 to 6) produced a paradoxical rise in vertebral number, while later pulses (at days 8 to 13) produced a fall in vertebral number. In neither species were data on completion times or control levels sufficient to allow trial of the atroposic model. For oxygen and other environmental factors also known to be able at sustained levels to modify vertebral numbers, no published experiments were available on effects of changed levels during development.

Fin-Ray Series

Responses of fin-ray counts to temperature breaks or pulses are recorded in several of the publications already cited with respect to vertebral counts. Others are described by Orska (1963, on *S. gairdneri*) and by Molander and Molander-Swedmark (1957, on *Pleuronectes platessa*). Both overcompensation and paradox can occur. For several fin-ray series in several species we have been able to fit the present version of the atroposic model. But the model will not fit some published series, and some of our own unpublished data. A few of the fin-ray series which cannot be fitted show an asymmetry in the patterns of response to temperature breaks applied in opposite directions. Perhaps use of the moment of fertilization as the origin of the atroposic curves is inappropriate, because the site along the body axis at which fin rays first form does not exist until some time after fertilization. Attempts to fit the atroposic model to fin rays using a starting time later than zero, or using L- and A-curves with mathematical forms more complex than the exponential, have been inconclusive

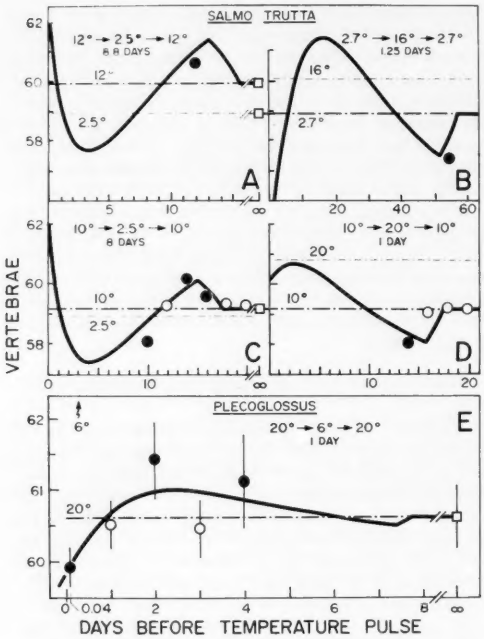

FIG. 5. Vertebral means in two species resulting from temperature pulses: developing embryos were moved from first to second indicated temperatures starting at times shown along horizontal axis, and then returned to first temperature after the number of days indicated by the label. Other symbols as in Fig. 4.

because of inadequate experimental data. Since some fin-ray series conform to the model that works for vertebrae, and some do not, we suspect that similar principles are involved but sometimes with added complexities.

Discussion

The atroposic model offers a fairly simple framework within which to fit an array of previously disparate experimental results. While it fits most available information on vertebrae of fishes, it may still be only an approximation of a more general model yet to be developed. A general model should provide coherence for other meristic series and perhaps other organisms; it should incorporate the concepts of experimental embryologists concerning mechanisms of pattern formation (e.g. how the *L*-process is translated into number of parts). Bearing in mind the limitations to what is being claimed for the present atroposic model, its strengths and weaknesses are discussed in the following section.

ADVANTAGES OF THE MODEL

The model requires few parameters to fit complex experimental results (Fig. 2, 4, 5). At each temperature in Table 1, vertebral count and completion time are based on observation, so only the two parameters λ_1 and α_1 need to be calculated (the other two, λ_2 and α_2, being thereby automatically constrained). For breaks and pulses between two temperatures, only two pairs of parameters need be calculated which generate all the curves to fit all the series of experiments.

As explained under Procedures, when experimental data are available both from break and pulse experiments, parameters can be calculated from just one set of data, and the model tested by using these parameters to predict the results of the other type of experiment. Such tests have added credence to the model using data for *S. trutta* and to a lesser extent for *S. gairdneri* and *R. marmoratus*, but no blocks of experiments have yet been performed specifically for this purpose.

For more than two temperatures, each parameter seems to vary with temperature in a regular fashion, forming a smooth family of curves (Fig. 3B) even when the response of vertebrae to sustained temperatures is sharply inflected (Fig. 2B). The atroposic hypothesis for *V*-shaped response is therefore simpler than other published hypotheses. The two arms of the response do not require the postulation of two unspecified mechanisms each dominant at one temperature extreme (Lindsey 1954), nor of a "most economical metabolism" at intermediate temperature (Marckmann 1954, criticized by Barlow 1961), nor of differential mortality, nor of miscounted complex vertebrae at temperature extremes (Garside 1966a). The radically different responses to sustained temperatures in different species (acclivous, or declivous, or V-shaped, or (in some fin rays) arched) can all be fitted by the same general model. Even within one species (the medaka), young from different parents may display either declivous or V-shaped vertebral responses (Ali and Lindsey 1974); such seemingly basic differences require only modest parameter changes in the one atroposic model. In all these cases the atroposic curves have an underlying regularity not readily apparent in the experimental data.

The model suggests that vertebral numbers may respond strongly and in either direction to environmental perturbations, which in fact they do. The model further suggests how these responses can be extralimitary, and how responses can be in opposite directions depending on the time of temperature change, and how large responses can be produced by quite brief temperature pulses. Previous discussions of these phenomena have involved from one to three "sensitive periods" during development, each thought to have a characteristic vertebral response to temperature change (Tåning 1952; Orska 1956; Fowler 1970). The atroposic model, on the other hand, implies that vertebral number is sensitive to environmental influence throughout the entire period from fertilization until final fixation of count. There is no profit in naming separate sensitive periods whose boundaries and directions of response depend on what temperature treatment is applied. (In *S. trutta* starting at 10°C, there will be one period of overcompensation followed by one of paradox if breaks to a lower temperature are applied (Fig. 4D), but there will be two paradoxes separated by one overcompensation if pulses are applied (Fig. 5C)).

Accompanying the older concept that there were discrete "sensitive periods," was the suggestion that during these times a sudden temperature change would produce a "shock

effect" on vertebral number (Tåning 1952). Extralimitary vertebral counts produced by temperature breaks or pulses were thought of as responses (metabolic upset?) to temperature change per se. Confusion ensued (Lindsey and Ali 1965: 103) in trying to interpret the results of temperature pulses or of continually alternating temperature, where the "shock effects" of successive temperature changes in opposite directions might cancel each other. In the atroposic model no shock from temperature change as such is invoked; all extralimitary responses are attributed to the cumulative and irreversible effect of the temperature regime on the progress of the *L*- and *A*-curves. It should be added that while no evidence exists that the rate of change during the temperature transfer makes a difference, there has been no experimental examination of the possibility.

Imperfections of the Model

If final vertebral number were solely determined by embryo length at time of vertebral fixation, then according to the atroposic model larger eggs (which produce longer embryos) should produce higher vertebral counts. They do not. Lindsey and Ali (1971) reviewed evidence that within a race of fish there is no causal connection between vertebral count and egg size. C. C. Lindsey and G. B. Ayles (unpublished data) using 36 reciprocal crosses of rainbow trout also found no correlation between egg size and vertebral number. Apparently there must be some degree of "regulation," the embryological term which implies an adjustment of internal spacing of segments in accordance with the total space available.

D. P. Swain has suggested to us that, in fish, the lack of correlation between vertebral count and initial embryo length may be because the *L*-process represents the number of cells rather than the length of undifferentiated tissue. Regulation might operate through the production of smaller cells in embryos of shorter initial length, but always with the same cell number in each early somite. The atroposic model will produce similar results regardless of whether the *L*-process stands for increase in absolute length or increase in cell number.

In amphibians, the early stages of somite formation seem to be regulative while the later stages are not. Larvae of *Xenopus* which had been reduced in size by early surgical removal of part of the blastula had somite numbers similar to those of normal-sized controls at the same developmental stage (Cooke 1975). On the other hand, late surgical removal of the tail bud tip did not result in regulation; the remaining somites formed were fewer in number although similar in size to the somites in controls (Cooke 1975, 1977). Perhaps in teleosts an initial regulation of the size of each somite may be dictated by egg size, while the ultimate number of somites is determined as envisioned by the atroposic model. In graphical terms, the spacing of the scale of the *L*-process would depend on early regulation, while the height reached on this scale would be determined by the completion time of the *A*-process. In the absence of surgical experiments on teleosts, this synthesis of the atroposic model with observations on amphibian morphogenesis is plausible but speculative.

Two implications in the atroposic model are questionable — that all segments are formed at one instant, and that all have equal spacing. In fact the determination of the boundaries between successive segments almost certainly occurs over an extended time, and the later-formed posterior segments are smaller than the early ones when they first appear. However, the assumptions are defensible as a mathematical artifice: spacing along the *L*-axis is not intended to represent physical distance in the embryo, but simply a scale (probably nonlinear) indicating the total number of segments which would be formed if completion occurred at that time. Nevertheless, some error may be introduced in analyzing results of temperature changes by the assumption that the same absolute increment in the *L*-process, regardless of where it occurs along the curve, will have the same effect on final vertebral count. The model cannot be refined in this respect until times and measurements are available for segments developing at different temperatures. At present, although some schedules are available showing times to reach defined embryonic stages at various temperatures (e.g. Hallam 1974), there are no comparable data on lengths of embryos or of somites.

No experimental data on meristic responses to temperature changes are extensive enough to distinguish between the suggested model and certain alternative mathematical treatments. Hence while the general class of model seems satisfactory, its details remain questionable. The failure of the present form of model to fit responses of some fin-ray series remains a serious imperfection.

Recommendations for Future Experiments

The very early temperature history of fertilized eggs used in meristic experiments needs to be better controlled. In most published experiments on effects of two or more sustained rearing temperatures, the eggs have been transferred to their respective temperature baths only after an appreciable (and often unspecified) time lapse following fertilization. That early transfers may produce significant meristic responses is suggested by our atroposic simulations, and was actually demonstrated in *Plecoglossus* (Komada 1977a). Experimenters should in future place eggs in their destined temperatures as soon as possible after fertilization. A series of tests could be run on the effects of various periods of delay. In fish such as *R. marmoratus*, the delay in egg extrusion until several hours following fertilization introduces doubt as to the interpretation of meristic counts at different "sustained" rearing temperatures (Lindsey and Harrington 1972; Harrington and Crossman 1976). Parental holding temperature may also affect meristic outcome not only because of the early temperature break inevitably applied to those batches of fertilized eggs destined for a different temperature; the prefertilization parental temperature may also directly affect offspring counts (Swain 1979). All these variables require better control and documentation than they have received in the past.

Temperature pulse experiments probably provide the most rigorous tests for meristic models. Unfortunately few such data have been published. Series of pulses applied at different developmental stages are needed, and series of pulses of different durations. In these as in all meristic experiments, genetic variation must be kept to a minimum, by using either a single fecund female for the whole block of experiments or an interlocking set of controls between different genotypes.

There is an obvious need for observational data on the dimensions and time of appearance of segments in fish

embryos at different sustained or transferred temperatures. Such a schedule might refute the interpretation of the *L*-process as embryonic elongation; in any event it should provide better insight into the mechanisms underlying meristic variation.

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Appendix A. Forms and Properties of the *L*-Curve and *A*-Curve

The forms chosen for modeling the *L*- and *A*-processes were those of exponential growth curves constrained to pass through the origin. Exponential forms are frequently found to give good descriptions of biological growth processes, at least over short periods of time. Moreover, they were found to be the simplest curves that could give rise to the extralimitary responses clearly evident in some break experiments. We originally modeled using logistic curves, which often occur in chemical kinetic processes (Kacser 1957). However, in fitting logistic curves to data we discovered it was necessary to use a cutoff point c below the inflection point on the logistic, so that the curves were essentially exponential. The possibility remains that logistic curves might be more satisfactory than exponential curves for some types of data which have been difficult to fit, such as responses of fin-ray counts. Perhaps the best generalized atroposic model follows logistic curves, and we deal here only with special cases in which only the lower portions of the curves are involved. Logistic curves are somewhat more difficult than exponential curves to fit by iteration.

In the exponential form of the *A*-process, we define $A(t; \alpha_1, \alpha_2)$ as the proportional commitment towards a process (possibly cell number, or concentration of a substance) which will trigger vertebral formation when it reaches the level c, given that the embryo has been reared for time t at a constant temperature. The parameters α_1 and α_2 determine the shape of the curve, and are temperature specific (that is, for rearings at two different temperatures the A curves will have differing parameter sets). The exponential form is

$$(1) \quad A(t) = A(t; \alpha_1, \alpha_2) = \alpha_1(\exp[\alpha_2 t] - 1)$$

All curves of this form have the following properties, so long as both parameters (α_1 and α_2) are positive valued:

(a) $A(0; \alpha_1, \alpha_2) = 0$ (i.e. the curve passes through the origin).

(b) $A(t)$ is monotone increasing in t.

(c) α_1 is a vertical scale parameter; that is, it simply determines the units for the vertical axis of the graph of $A(t)$ vs. t. As a consequence, the level c (called the completion level) is arbitrary, and has been set at 10 for all model fits.

(d) α_2 is a shape parameter: for given α_1, a curve with smaller α_2 value will rise less quickly and have less curvature than a curve with larger α_2. All $A(t)$ curves are concave upwards (Fig. 3)

(e) As a consequence of (b), the time taken to reach any specified commitment level, a, is uniquely determined. This time is obtained by setting the right-hand side of equation (1) to the level a and solving for t. Let $TA(a; \alpha_1, \alpha_2)$ be the time to reach a given level a; then:

$$(2) \quad TA(a; \alpha_1, \alpha_2) = (1/\alpha_2)\log[(\alpha_1 + a)/\alpha_1]$$

Turning to the exponential form of the *L*-process, we define $L(t; \lambda_1, \lambda_2)$ as the (expected) number of vertebrae that would be formed at time t if completion were to occur t units after fertilization, given that it was reared at constant temperature. The parameters λ_1 and λ_2 are, again, temperature specific, and the *L*-curve is of the exponential form:

$$(3) \quad L(t; \lambda_1, \lambda_2) = \lambda_1 (\exp[\lambda_2 t] - 1)$$

Thus properties (a) to (e) above also hold for the *L*-curve at any given temperature,and in particular, the time taken to reach a given level ℓ of the *L*-process is given by:

$$(4) \quad TL(\ell; \lambda_1, \lambda_2) = (1/\lambda_2)\log[(\lambda_1 + \ell)/\lambda_1].$$

Appendix B. Derivation of Expected Outcome of Experimental Manipulations

If an embryo is reared at a specified constant temperature (i.e. α_1, α_2, λ_1, and λ_2 are specified), then the final vertebral number is determined when the *A*-process reaches the completion level, c. If we denote this "completion time" by t_c, then from equation (2):

$$(5) \quad t_c = TA(c; \alpha_1, \alpha_2) = (1/\alpha_2)\log[(\alpha_1 + c)/\alpha_1].$$

If completion takes place at time t_c, the final vertebral number, say V_c, is given by equation (3) with $t = t_c$

$$(6) \quad V_c = \lambda_1(\exp[\lambda_2 t_c] - 1).$$

The model was not used, however, to "explain" the response of t_c and V_c to different (constant) rearing temperatures; rather, equations (5) and (6) were used to constrain the model to satisfy observed t_c and V_c values. The method used is described briefly in Appendix C, and in more detail in Arnason et al. (1978).

In a break experiment such as illustrated in Fig. 1, embryos are reared at one temperature, say T_{lo}, up to time t_s, and are then shifted to a second temperature, say T_{hi}, and reared until (long) after the vertebral number is determined. The model can be used to predict the resulting vertebral number, V_s, say, given the A and L curve parameters for constant rearing both at low temperature ($\alpha_1(T_{lo})$ and $\alpha_2(T_{lo})$ for A; $\lambda_1(T_{lo})$ and $\lambda_2(T_{lo})$ for L) and at high temperature ($\alpha_1(T_{hi})$, $\alpha_2(T_{hi})$, $\lambda_1(T_{hi})$, $\lambda_2(T_{hi})$). The parentheses after the parameters denote that the parameters at the two temperatures must differ, since for both the A- and the L-process it is reasonable to assume that the process always accelerates more quickly at the higher temperature. Thus the higher temperature A-curve must lie entirely above the lower temperature A-curve except at the origin (Fig. 1, 3). A sufficient condition for this "monotonicity" property to be true is that:

$$(7a) \quad \alpha_2(T_{hi}) > \alpha_2(T_{lo})$$

and

$$(7b) \quad \alpha_1(T_{hi})\cdot\alpha_2(T_{hi}) > \alpha_1(T_{lo})\cdot\alpha_2(T_{lo}).$$

Similarly, sufficient conditions for the higher temperature

L-curve to lie above the lower temperature L-curve are that:

(8a) $\lambda_2(T_{hi}) > \lambda_2(T_{lo})$

and

(8b) $\lambda_1(T_{hi})\cdot\lambda_2(T_{hi}) > \lambda_1(T_{lo})\cdot\lambda_2(T_{lo})$.

These conditions are sufficient but not necessary; there may be parameter sets which fail the conditions yet the monotonicity property still holds. In practice, these conditions are quite strong; they were always satisfied by the parameters obtained from all fits to break or pulse experiments, as can be seen in Table 1.

Given parameters that satisfy these conditions, the result of a break from T_{lo} to T_{hi} ($T_{hi} > T_{lo}$) at time t_s has the following properties:

(a) if $t_s = 0$, the experiment is equivalent to constant rearing at T_{hi}, and $V_s = V_{hi}$, (see Fig. 1).

(b) if $t_s \geq t_c(T_{lo})$ (which is the completion time at T_{lo}), as computed from equation (5) using $\alpha_1(T_{lo})$ and $\alpha_2(T_{lo})$, then the experiment is equivalent to constant rearing at T_{lo}, and $V_s = V_{lo}$ (see Fig. 1).

(c) Otherwise, ($0 < t_s < t_c(T_{lo})$):

At time t_s, the embryo has evolved along the low temperature A-curve to the level a_s (using equation (1) with $t = t_s$)

(9) $a_s = A(t_s; \alpha_1(T_{lo}), \alpha_2(T_{lo}))$.

and along the low temperature L-curve to the level l_s (using equation (3) with $t = t_s$)

(10) $l_s = L(t_s; \lambda_1(T_{lo}), \lambda_2(T_{lo}))$.

Levels a_s and l_s are shown in Fig. 1.
The time to reach level $a = a_s$ at high temperature is found using equation (2), and is:

(11) $t_a = TA(a_s; \alpha_1(T_{hi}), \alpha_2(T_{hi}))$

and completion time at high temperature is (from equation (5)):

(12) $t_{hi} = TA(c; \alpha_1(T_{hi}), \alpha_2(T_{hi}))$.

Hence the time left after t_s until completion is:

(13) $t_d = t_{hi} - t_a$.

But the time taken to reach the level l_s at high temperature would be (using equation (4)):

(14) $t_\ell = TL(l_s; \lambda_1(T_{hi}), \lambda_2(T_{hi}))$

and the embryo continues to evolve along the high temperature L-curve for a further t_d time unit, at which time the final vertebral number is determined as (using equation (3) with $t = t_\ell + t_d$):

(15) $V_s = L(t_\ell + t_d; \lambda_1(T_{hi}), \lambda_2(T_{hi}))$.

Note that the results given for this break experiment in equations (9)−(15) are perfectly general. They apply to any family of A- and L-curves, not just for the exponential form, and with only a change of notation, could be extended to families characterized by more than two parameters. There is no requirement that A and L be of the same form; the essential properties are simply monotonicity with respect to time (for each member of each family) and with respect to temperature (among members of each family). For the exponential forms of A and L defined in Appendix A, the numerical value of V_s is obtained, for given parameter values and shift time, t_s, by the process of successive substitutions defined in equations (9)−(15). The following worked example may help to clarify this process:

For a break from $T_{lo} = 20$°C to $T_{hi} = 30$°C at time $t_s = 8$ (d) for *Oryzias latipes*, the required parameter substitutions are, from Table 1:

$\alpha_1(T_{lo}) = 4.7902$ $\alpha_2(T_{lo}) = 0.1084$ $\lambda_1(T_{lo}) = 0.4244$ $\lambda_2(T_{lo}) = 0.4148$

$\alpha_1(T_{hi}) = 6.0020$ $\alpha_2(T_{hi}) = 0.2086$ $\lambda_1(T_{hi}) = 0.5092$ $\lambda_2(T_{hi}) = 0.8763$

and the completion level for the A curves is $c = 10$, thus

$$a_s = 4.7902\ (\exp[(0.1084)(8)] - 1) = 6.6116$$

$$l_s = 0.4244\ (\exp[(0.4148)(8)] - 1) = 11.296$$

$$t_a = (1/0.2086)\log[(6.0020 + a_s)/6.0020] = 3.5603$$

$$t_{hi} = (1/0.2086)\log[(6.0020 + c)/6.0020] = 4.7010$$

$$t_d = 4.7010 - 3.5603 = 1.1406$$

$$t_\ell = (1/0.8763)\log[(0.5092 + l_s)/0.5092] = 3.5872$$

$$t = t_\ell + t_d = 4.7278$$

$$V_s = 0.5092\ (\exp[(0.8763)(4.7278)] - 1) = 31.565.$$

It is necessary to carry a large number of significant figures in the intermediate calculations as round-off error builds up quickly; 16 figures were carried at each step above, but results are shown to only five significant figures. Plots of the break response are obtained by repeating this computation using the same parameter values but for a series of t_s values.

Note that V_s completes at time $t_s + t_d$, and that the series of V_s values in Fig. 1 is plotted against this completion time for a series of t_s values. When the model is fitted to real data (Fig. 4), the actual completion time of a break experiment is not observed, so V_s is plotted against t_s.

If the break is in the opposite direction, from T_{hi} to T_{lo}, then the arguments given in (a), (b), and (c) above apply exactly if T_{hi} is substituted for T_{lo} and vice versa.

It can be shown that, by appropriate choice of parameter values, this break model may produce both types of extralimitary response, or paradoxical responses only, or overcompensations only, or neither (V_s always lying between V_{lo} and V_{hi}). We have always found that the types (although not the degree) of extralimitary response in a break in one direction (say

T_{lo} to T_{hi}) are also present in the reciprocal break response (say T_{hi} to T_{lo}) (Fig. 4), but we have not been able to prove that this is a necessary mathematical consequence of the model.

We shall now analyze a pulse experiment, in which embryos are reared at one temperature, say T_{lo}, up to time t_s, and then shifted to the second temperature, T_{hi}, for a further time τ, and then (at time $t_s + \tau$) are shifted back to T_{lo} to complete rearing. As with break experiments, the parameters for two sets of A- and L-curve giving the effects of rearing at constant T_{hi} and T_{lo} are needed, and these must satisfy the monotonicity requirements (equations (7) and (8)). Given these, the results of the pulse for given t_s and τ can be calculated as follows:

(a) if $t_s = 0$, the experiment is equivalent to a break from T_{hi} to T_{lo} at time $t = \tau$.

(b) if $t_s + \tau > t_s + t_d$ where t_d is calculated using equation (13), then the experiment is equivalent to a break from T_{lo} to T_{hi} at time $t = t_s$ (because vertebral number is fixed while the embryo is at T_{hi} and the second temperature shift has no effect).

(c) Otherwise: the effect of the pulse can be worked out using an argument similar to that in (c) preceding, except the mapping between the low and high A-curves (equations (9) and (11)) and L-curves (equations (10) and (14)) must be repeated an extra time in the opposite direction (from T_{hi} to T_{lo}) to model the effect of the second shift at time $t_s + \tau$. A more detailed description is given in Arnason et al. (1978).

Pulses in the opposite direction (from T_{hi} to T_{lo} to T_{hi}) are modeled by carrying out the calculations described in (a) to (c) above with T_{lo} replaced by T_{hi} and vice versa. The pulse response curve, obtained by plotting the pulse response V_s versus the first shift time t_s for fixed τ ($t_s = 0 \ldots t_c(T)$ where $t_c(T)$ is completion time for constant rearing at the initial temperature), generally shows one local maximum and one local minimum, plus possibly a local maximum or minimum at $t_s = 0$; the latest of these local extrema is a cusp (discontinuity) where the pulse degenerates to a break (at $t_s + \tau = t_s + t_d$). Figure 5 illustrates typical results, but more complex outcomes are possible with certain parameter values.

Appendix C. Fitting the Model to Experimental Results

To fit parameters of the model to response data from break and/or pulse experiments, we have developed an interactive graphics package written in APL computer language and used with a Tektronics 4002A display terminal and 4601 hardcopier (Arnason et al. 1978). The system is interactive with the user in the sense that a series of prompts are displayed, each requiring the entry of an appropriate response. The results of calculations can be displayed either as digits or as graphs; the latter are the sources of Fig. 3–5 here. For fitting, at least eight mean vertebral counts are required at distinct shift times, t_s, or for different experimental types or directions, but using only two temperatures. (Because our particular program required eight experimental points, it was necessary when fewer were available (e.g. for *Plecoglossus*) to add guessed points which were later discarded after optimization.) Fitting is done using nonlinear least squares, employing Marquardt's (1963) algorithm to find parameters that minimize the weighted sum of squared residuals between the observed means and the model prediction. This method could have been used to find all eight parameters ($\alpha_1(T)$, $\alpha_2(T)$, $\lambda_1(T)$, and $\lambda_2(T)$ for both $T = T_{lo}$ and $T = T_{hi}$) but the algorithm would be too slow to be practical. Since the constant rearing experiments at T_{lo} and T_{hi} give reliable values for V_{lo} and V_{hi}, and since reasonably good completion times at constant rearing are available from break experiments (or can be estimated from comparable stages of related species), equations (5) and (6) can be used to reduce the number of parameters that must be fitted to four. That is, the least squares algorithm is used to find values of $\alpha_1(T_{hi})$, $\alpha_1(T_{lo})$, $\lambda_1(T_{hi})$, and $\lambda_1(T_{lo})$, and then $\alpha_2(T_{hi})$ and $\alpha_2(T_{lo})$ are obtained by solving equation (5) for α_2 given completion times $t_c(T_{hi})$ and $t_c(T_{lo})$, respectively. Similarly, equation (6) is used to obtain $\lambda_2(T_{hi})$ and $\lambda_2(T_{lo})$ for given completion times $t_c(T_{hi})$, $t_c(T_{lo})$ and constant rearing levels $V_c(T_{hi})$, $V_c(T_{lo})$, respectively. The formulae are easily derived from equations (5) and (6). This substitution is, in fact, carried out at each step of the iterative least squares procedure each time the algorithm selects new $\alpha_1(T_{hi})$, $\alpha_1(T_{lo})$, $\lambda_1(T_{hi})$, and $\lambda_1(T_{lo})$ parameters in a directed search for those values that minimize the sum of squared residuals. Thus at each step, the model predicts the observed t_c and V_c values exactly at both T_{hi} and T_{lo}. The optimization algorithm is carried out without constraints on the four parameters, so after the algorithm has converged it is important to check that the monotonicity conditions (equations (7) and (8)) are satisfied by the final (fitted) parameter values. The fact that this was always found to be the case for the model fits carried out in this paper is a further indication of the biological realism of the model. The average running time for optimization was roughly 20 s elapsed time, but probably only a few seconds of computer (CPU) time.

Differential Response to Water Current by Two Homozygous LDH Phenotypes of Young Rainbow Trout (*Salmo gairdneri*)

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NORTHCOTE, T. G., and B. W. KELSO. 1981. Differential response to water current by two homozygous LDH phenotypes of young rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 38: 348–352.

Above-waterfall, wild stocks of rainbow trout fry homozygous for liver lactate dehydrogenase alleles ldh $H\alpha^A$ and ldh $H\alpha^B$ were artificially propagated, and their response to water current was compared in experimental channels permitting upstream or downstream movement. Although $H\alpha^A$ $H\alpha^A$ phenotypes showed significantly more upstream movement than $H\alpha^B$ $H\alpha^B$ phenotypes in tests made under lighted conditions and less downstream movement in darkness, these differences in behavior cannot necessarily be ascribed to the ldh $H\alpha$ locus. Implications to control of current responses in trout maintaining populations above waterfalls impassible to upstream migration are discussed.

Key words: current response, LDH, rainbow trout, migratory behavior

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Dans l'étude décrite ici, on a propagé artificiellement, en amont d'une chute, des stocks sauvages d'alevins de truite arc-en-ciel homozygote pour les allèles de lactate déshydrogénase hépatique ldh $H\alpha^A$ et ldh $H\alpha^B$ et comparé leur réaction à un courant d'eau dans des chenaux expérimentaux permettant des déplacements vers l'amont et vers l'aval. Les phénotypes $H\alpha^A$ $H\alpha^A$ se déplacent plus souvent vers l'amont que les phénotypes $H\alpha^B$ $H\alpha^B$ dans des essais effectués à la lumière et moins souvent vers l'aval à la noirceur. Cependant, on ne peut attribuer ces différences de comportement nécessairement au locus ldh $H\alpha$. Nous analysons les implications du contrôle des réactions à un courant chez des truites arc-en-ciel qui maintiennent une population en amont de chutes bloquant la migration vers l'amont.

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NORTHCOTE (1969) suggested that genetically controlled differences might occur in current responses of trout and char populations living above and below waterfalls in several British Columbia stream systems and presented some field evidence for such behavior in rainbow trout (*Salmo gairdneri*) inhabiting Kokanee Creek, a tributary to Kootenay Lake. The existence of meristic and lactate dehydrogenase (LDH) genotypic differences were then demonstrated in this population and the plan to compare experimentally the swimming endurance and current responses of homozygous LDH phenotypes was outlined (Northcote et al. 1970). Results of experiments on swimming endurance of homozygous LDH juveniles from another above-waterfall population (Loon Lake) already have been reported (Tsuyuki and Williscroft 1977); those on current responses of fry from the same population and crosses are given herein.

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Material and Methods

EXPERIMENTAL STOCKS

Rainbow trout adults utilized to propagate the appropriate crosses came from the main inlet to Loon Lake in the southern interior of British Columbia. This lake contains a native population of rainbow trout which spawn both in the inlet stream and in its outlet above a waterfall impassible to upstream movement of fish (Lindsey et al. 1959; Hartman et al. 1962).

Methods used to obtain the two homozygous LDH types of adults for setting up the crosses are summarized by Tsuyuki and Williscroft (1977). Forty-one adults were seined from the main inlet stream to Loon Lake and the LDH phenotypes determined from sera analysis. The adult LDH types were identified by finclips and held at Loon Creek Hatchery until they reached full sexual maturity (water temperature, 9–10°C). Because of mortality during the holding period of about 1 mo and problems in getting sufficient ripe adults of the appropriate LDH type at any one time to propagate the crosses, the number of parents used was small. In total, 2 $H\alpha^A$ females and 3 $H\alpha^A$ males were used to propagate the

$H\alpha^A$ $H\alpha^A$ crosses[2] and 4 $H\alpha^B$ females and 6 $H\alpha^B$ males the $H\alpha^B$ $H\alpha^B$ crosses which yielded ~ 1250 $H\alpha^A$ $H\alpha^A$ and 2500 $H\alpha^B$ $H\alpha^B$ eggs (Table 1). For crosses where several males and females were used, eggs and sperm were pooled separately and mixed before fertilization was effected. Young from these crosses were pooled and reared first at the Loon Creek Hatchery and then later at the Fraser Valley Hatchery, Abbotsford, in indoor troughs at water temperatures close to 10°C, using a standard hatchery dry meal diet.

TABLE 1. Parental genotype of adults used to propagate homozygous LDH phenotypes of rainbow trout fry for current response experiments.

Date of cross	Genotype	Parental males	Number females	Fertilized eggs
June 25	$H\alpha^B$	4	3	2000
June 29	$H\alpha^A$	1	1	500
July 18	$H\alpha^B$	2	1	500
July 22	$H\alpha^A$	2	1	750

CURRENT RESPONSE CHANNELS

Current response tests were performed at Rosewall Creek Hatchery, Vancouver Island, on the fry placed in channels which permitted them to move either upstream or downstream through a series of small pool-like compartments (Fig. 1). Wooden troughs ~ 15 cm in cross section and 3 m long were divided into 25 equal-sized compartments, interconnected by staggered holes 2.6 cm in diameter. Thus, a fish moving either upstream or downstream from one compartment to another could not do so directly but had to "seek out" the entrance of the next opposing one. Small V-shaped traps in compartments at each end of the channel retained fish which had moved that far from the central release compartment. Twelve such channels were used in two banks of six.

A darkroom ~ 3.7 m × 3.7 m × 2.7 m, was constructed by covering a wooden framed area with 4-mil black polyethylene plastic. It was divided by a polyethylene partition into two rooms, each having a separate entrance and containing a bank of six current response channels. All 12 channels were supplied with water from the same headtank which protruded through the center partition into each room. Controlled flow (1.25 L/min) was maintained to each channel giving a velocity of 7.4 ± 0.5 cm/s through the comparment openings.

The illumination used for light tests with each bank of current response channels consisted of five 150-W, 125-V, projector flood lamps. These were hung on the center partition wall and directed upward onto a white glossy ceiling to give indirect illumination eliminating shadows in the channels. Tests in darkness were run with all lights inside the darkroom turned off. The lights were controlled from outside the room with an automatic timer and a powerstat voltage manipulator. The timer took ½ h to increase voltage to maximum (115 V) or decrease it to minimum (0 V). All tests were made with water close to 9°C (minimum 9.0, maximum 9.8) and never varied more than 0.4°C between the start and end of any one test.

TEST PROCEDURE

Prior to testing, fry of each stock were transported to Rosewall Creek Hatchery and held separately in tanks at ~ 9°C. Twenty fry, 30–35 mm long, were used in each of the 12 channels where replicate tests were run. Fry to be tested were dipnetted from the holding tanks and placed in the central compartment of each current response channel. For tests in the light, the experiments were begun in darkness and the lights were increased slowly by the automatic timer to maximum intensity over a ½-h period. Fifteen minutes after the test was begun (light intensity about half maximum), the screens on the exit holes were removed and the fry were free to move upstream or downstream a maximum of 12 compartments in either direction. Those in the dark were done in the same manner except that the lights were slowly decreased to zero intensity by the timer over the same time interval. Each test was terminated 2¼ h after release time and the number of fry in the 25 compartments recorded.

CURRENT RESPONSE SCORING

A "rank number" scoring system was developed for analysis of fry response to water current. This system assigned a rank number to each compartment starting with the central compartment as zero. Those upstream or downstream from it were numbered 1 to 12 consecutively (Fig. 1). The number of fish per compartment was multiplied by its rank number, summed, and then divided by the total number of fish that were used in the test. To maintain positive scores, a constant of 12 was added. This scoring system would not readily distinguish between cases where all fry remained in the central compartment (upstream and downstream score = 12) and those where all of the fry moved either (a) down into the lowermost compartment or (b) up into the uppermost compartment [(a) score = 12 for upstream movement and 24 for downstream movement, (b) score = 24 for upstream movement and 12 for downstream movement]. In no instance, however, did such fry distributions arise during the 48 separate current response tests performed.

Results

The data on fry distribution in the current response channels after tests on the two LDH phenotypes clearly suggest differences between tests in light and dark (Table 2). The average number of fry moving the maximum distance upstream (compartment 12) in the channel was much higher in the light than in the dark. On the other hand, averages for maximum downstream movement were much higher in the dark than in the light.

Of more interest is the clear evidence for differences in current response between the two LDH phenotypes tested under the same conditions. The average number of fry moving the maximum distance upstream in the channels is over two times higher in the $H\alpha^A$ $H\alpha^A$ homozygote compared to the other form whether tested under light or dark conditions. In the 12 tests in the light, as many as 15 and never fewer than 8 of the 20 $H\alpha^A$ $H\alpha^A$ ascended to the upermost compartment, whereas at most only half and sometimes as few as 1 of the

[2]The nomenclature of Bailey et al. 1976 is followed.

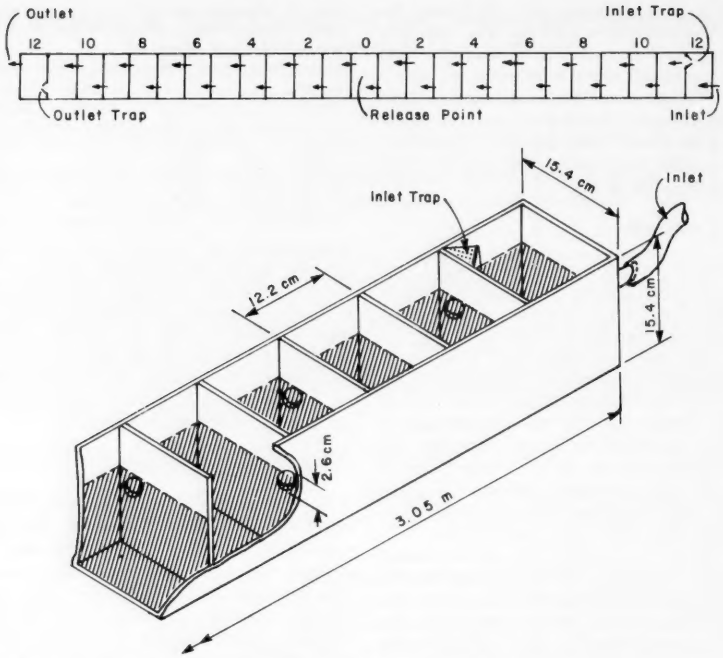

FIG. 1. The standard 25-compartment channel used to test response of rainbow trout fry to water current. Upper plan view shows location of central compartment where fry were released as well as numbers assigned to compartments for calculation of the rank number score for expressing current response. Lower sketch shows upper portion of the channel oriented to expose staggered openings between compartments.

$H\alpha^B$ $H\alpha^B$ fry reached the upstream compartment (Table 2). In contrast, the $H\alpha^B$ $H\alpha^B$ fry exhibited more downstream movement than did the $H\alpha^A$ $H\alpha^A$ fry whether tested in light or dark conditions.

To use a more sensitive means of summing directional response to water current by the young trout, the rank number scores for downstream movement were subtracted from those for upstream movement and a constant of 12 was added to prevent negative numbers. This "net" rank number score showed even more clearly the marked differences between light and dark tests as well as those between the two LDH forms (Fig. 2). An analysis of variance run on the net scores (Table 3) gave highly significant effects of stock and illumination on the response of the trout fry to water current in the channel tests. The first-order interaction (stock × illumination) also was significant; $H\alpha^A$ $H\alpha^A$ fry showed greater upstream movement than $H\alpha^B$ $H\alpha^B$ fry, especially in lighted conditions. Illumination accounted for nearly 73% of the variance observed.

Discussion

The predominantly negative current response (downstream movement) exhibited by the rainbow trout fry of Loon Lake inlet stock in tests made in the dark was expected and agrees with their behavior demonstrated previously in laboratory as well as field experiments at water temperatures close to 10°C (Northcote 1962). Lakeward (downstream) migration of this stock in the inlet stream occurs almost entirely at night with little or no movement, downstream or upstream, during the day unless water temperatures approach 15°C or more when there may be some upstream movement. Thus, the highly significant differences in current response of these fry shown between tests conducted under light and dark conditions (Fig. 2, Table 2) fit well with what is known of their migratory behavior in the field.

Of more interest is the finding that there are also significant differences in the response to water current shown by the two homozygous LDH phenotypes propagated from Loon Lake inlet stock. These same phenotypes, apparently under the control of a two-allele system (see also Bailey et al. 1976), were reported from above- and below-waterfall populations of rainbow trout in Kokanee Creek, British Columbia (Northcote et al. 1970). The homozygous form $H\alpha^A$ $H\alpha^A$ was predominant in the above-waterfall population of trout, whereas the other homozygous form $H\alpha^B$ $H\alpha^B$ was predominant in those from below the waterfall. It was speculated then that these LDH forms might well confer differences in swimming endurance and response to current which would be of adaptive significance to headwater trout populations subject to the

TABLE 2. Numerical response to water current in experimental channels by two homozygous LDH phenotypes[a] of rainbow trout fry. Data summarized for 12 separate tests of each stock in light and dark conditions.

		Number of fry recorded in compartments[b]									
		12 Upstream		1–11 Upstream		0 Central		1–11 Downstream		12 Downstream	
		A	B	A	B	A	B	A	B	A	B
Light	Max.	15	10	5	5	1	1	6	8	8	12
	Mean	11.3	5.0	2.3	2.8	0.1	0.1	2.1	4.9	4.3	7.3
	Min.	8	1	0	0	0	0	0	2	2	2
Dark	Max.	4	3	2	2	2	2	3	3	17	20
	Mean	2.2	0.9	0.8	0.3	0.3	0.3	1.7	0.8	15.1	17.6
	Min.	1	0	0	0	0	0	1	0	12	14

[a]A = $H\alpha^A$ $H\alpha^A$; B = $H\alpha^B$ $H\alpha^B$.
[b]See Fig. 1 for location of compartments in experimental channels.

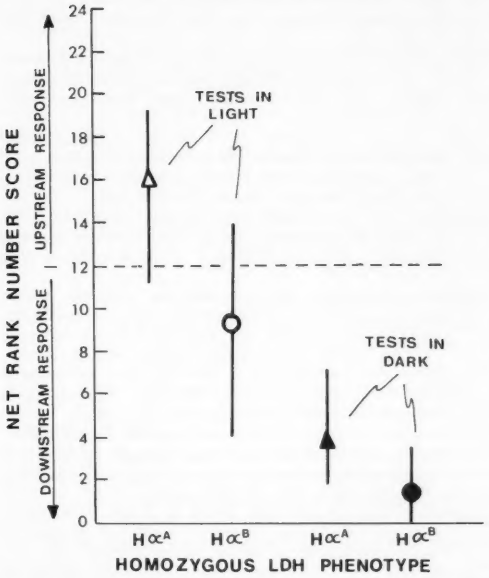

FIG. 2. Response to water current shown by two homozygous LDH phenotypes of rainbow trout fry in standard experimental channel tests. Symbols and vertical lines give means and ranges, respectively, for scores obtained for 12 replications of each test condition.

TABLE 3. Analysis of variance on net rank number scores for current response of two homozygous LDH stocks of rainbow trout fry in standard experimental channel tests with 12 treatment replications. $^{***}P < 0.001$; $^{**}P < 0.01$.

Factor	df	Variance	% Variance	F
Stock ($H\alpha^A$ $H\alpha^A$ or $H\alpha^B$ $H\alpha^B$)	1	9.66	13.71	44.93***
Illumination (light or dark)	1	51.38	72.87	234.56***
Stock × illumination interaction	1	4.18	5.93	10.50**
Error	44	5.28		

strong selective pressure for maintenance of position that presumably occurs in rapidly flowing streams above waterfalls. That the "above-waterfall type," $H\alpha^A$ $H\alpha^A$, occurs in both inlet and the outlet tributaries of Loon Lake (Huzyk and Tsuyuki 1974) is not surprising since these are all above the waterfall on the system. Furthermore, because of the disparity in numbers of spawners utilizing the inlet and outlet of the lake, even the low and similar percentage of straying by adult spawners originating from the two streams could result in up to a quarter of the outlet spawning run being composed of inlet strays (Lindsey et al. 1959).

Subsequently, it has been demonstrated that the above-waterfall type of LDH has a lower secondary narrow pH optimum in the physioligical range than that of the below-waterfall type (7.30 vs. 7.43); the former being 3.6 times more efficient in lactate to pyruvate conversion (Tsuyuki and Williscroft 1973; see also Kao and Farley 1978). This difference in lactate conversion rate between the two LDH forms does seem to be of physiological importance because homozygous rainbow trout juveniles (5–6 cm fork length) of the $H\alpha^A$ $H\alpha^A$ type exhibited 2.3 times greater swimming stamina (Tsuyuki and Williscroft 1977) when compared to $H\alpha^B$ $H\alpha^B$ counterparts of the same size from Loon inlet stock. The superior swimming endurance of the $H\alpha^A$ $H\alpha^A$ phenotype, however, decreased greatly after 2 yr of age and Klar et al. 1979 could not demonstrate any significant advantage in performance of this form using 29- to 32-cm sexually mature rainbow trout. Apparently there may be ontogenetic changes in swimming ability related to changes in physiological performance of the LDH phenotype as suggested by Klar et al. 1979. This possibility as well as that of ontogenetic changes in current response should be investigated further. An association between LDH genotype and the external environment of the killifish (*Fundulus heteroclitus*) mediated by the physiolo-

gical role of hemoglobin, has been shown by Powers et al. 1979 and suggests in another area, the selective advantage that LDH form may confer upon individuals competing in a rigorous environment.

Our observations suggest that there also may be behavioral differences between homozygous young with the two LDH types, those with the $H\alpha^A$ $H\alpha^A$ form showing a more positive response to water current. Of course, we do not claim on the basis of these experiments that the behavioral differences observed between the two LDH phenotypes were caused by the ldh $H\alpha$ locus. A much more elaborate and extensive series of experiments would be needed to support such an interpretation. Furthermore, we do not know that the gene system for the LDH forms which seem to effect stamina differences are the same or only ones involved in the current response differences. Nevertheless, there still remains the intriguing possibility that a genetically controlled enzyme system apparently altering the physiological capabilities of a fish may also be linked with a pattern of behavior such as current response in ways which would be of clear selective advantage, especially in maintenance of headwater stream populations above waterfalls.

It is unfortunate that difficulties in obtaining fully mature and appropriate LDH adult phenotypes did not permit us to test offspring based on a larger parental number. However, the experiments conducted by Tsuyuki and Williscroft (1977) on swimming endurance of young rainbow trout originating from the same crosses as ours did give results similar to those found in young with the same LDH forms which were propagated from a much larger parental number of Thompson River steelhead.

So far (Northcote et al. 1970; Williscroft and Tsuyuki 1970; Utter and Hodgins 1972; Huzyk and Tsuyuki 1974; H. Tsuyuki personal communication), the homozygous $H\alpha^A$ LDH form is common only in native rainbow trout from headwaters of two British Columbian systems: Kokanee Creek and Okanagan Lake (Columbia River watershed), Loon and Pennask Lake tributaries (Fraser River watershed). It does occur in similar homozygous proportions in anadromous rainbow trout (steelhead) of the Thompson River (Huzyk and Tsuyuki 1974), a population migrating several hundred kilometres up the Fraser River system past many sections of rapids where ascent must be difficult, but not in the lower Fraser nor coastal river populations. Furthermore, Allendorf (1975) has reported high frequencies of the $H\alpha^A$ phenotype to be present in steelhead from the upper Columbia system but much lower frequencies from streams tributary to its lower reaches or to small coastal drainages in Washington.

Acknowledgments

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Shifts In Aquatic Insect Populations In A First-Order Southern Appalachian Stream Following A Decade Of Old Field Succession

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HAEFNER, J. D., AND J. B. WALLACE. 1981. Shifts in aquatic insect populations in a first-order southern Appalachian stream following a decade of old field succession. Can. J. Fish. Aquat. Sci. 38: 353–359.

Aquatic insects of two first-order southern Appalachian streams were sampled monthly during one year. Sawmill Branch Watershed was subjected to several disturbances before 1968, including clear-cutting. Since 1968 Sawmill Branch Watershed has undergone natural secondary succession from terrestrial vegetation dominated by Gramineae to a herbaceous/coppice hardwood dominated by black locust, *Robinia pseudoacacia*. Grady Branch, the control stream, drains an undisturbed hardwood watershed. Aquatic insect densities on Sawmill Branch were about twice those of Grady Branch, which represent a dramatic reversal from results obtained by a similar study in 1968. It is suggested that change in riparian vegetation is a major factor influencing long-term changes in aquatic insect populations, and these changes are manifested through a shift toward an allochthonous energy base. The most conspicuous changes in macrobenthos on the disturbed watershed between 1968 and 1978 were a reduction in grazer organisms and a corresponding increase in shredder organisms, especially *Peltoperla* (Plecoptera).

Key words: macrobenthos, secondary succession, watershed changes, clear-cutting, allochthonous detritus, food quality.

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Nous avons prélevé mensuellement pendant un an des échantillons d'insectes aquatiques dans deux cours d'eau de premier ordre du sud des Appalaches. Le bassin hydrographique de la branche Sawmill a subi plusieurs perturbations avant 1968, y compris la coupe à blanc des bois. Depuis 1968, il y a dans ce bassin une succession secondaire naturelle, allant d'une végétation terrestre dominée par des graminées à un complexe herbacées/taillis de bois dur dominé par l'acacia blanc, *Robinia pseudoacacia*. La branch Grady, le cours d'eau témoin, draine un bassin de bois durs non perturbés. La densité des insectes aquatiques dans la branche Sawmill est environ le double de celle de la branche Grady. Ceci est tout à fait à l'opposé des résultats d'une étude semblable menée en 1968. Nous croyons qu'un changement dans la végétation riveraine est un facteur qui influe beaucoup sur les changements à long terme dans les populations d'insectes aquatiques, et que ces changements se manifestent par un glissement vers une base énergétique allochtone. Les changements les plus remarquables, entre 1968 et 1978, dans le macrobenthos du bassin perturbé ont été une diminution des organismes brouteurs et une augmentation correspondante des organismes broyeurs, particulièrement *Peltoperla* (Plecoptera).

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ALLOCHTHONOUS inputs of leaf detritus are the major energy source for benthic invertebrates in most heavily shaded first-order streams (Hynes 1970; 1975; Cummins 1974). Because of dependence on leaf litter and its associated microflora as a food source (Cummins 1973), the distribution of many benthic invertebrates is correlated with that of leaf detritus (Egglishaw 1964; G. W. Minshall 1967; Woodall and Wallace 1972). Other factors including current velocity and substrate composition affect the distribution of benthic invertebrates and detritus (Ulfstrand 1967; Cummins and Lauff 1969; Minshall and Minshall 1977; Rabeni and Minshall 1977).

Woodall and Wallace (1972) investigated benthic insect

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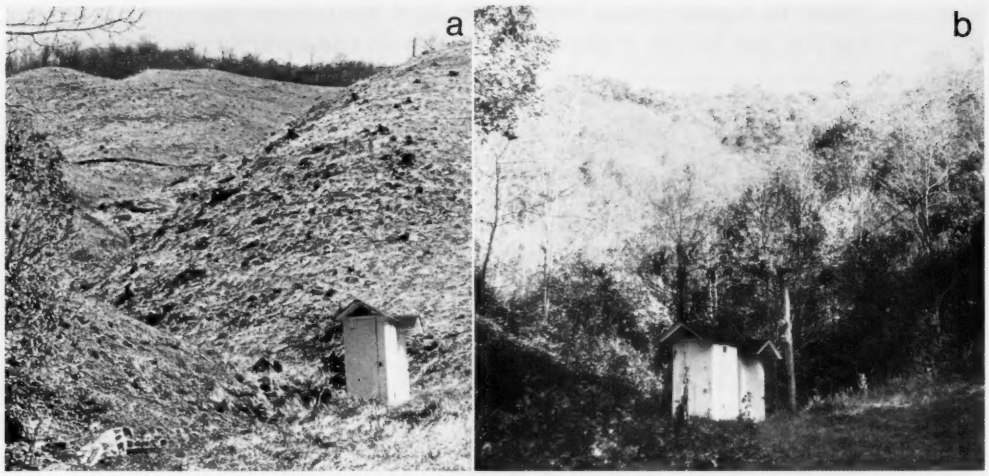

FIG. 1a and b. Sawmill Branch Watershed (WS 6) facing upstream from the weir. Left, watershed as it appeared in the spring of 1968. Right, same at slightly different angle, as it appeared in autumn, 1978, following 10 years of natural secondary succession.

communities in four first-order southern Appalachian streams, including hardwood and old-field watersheds. They associated differences in species composition and abundance between the streams, with varying quantity and quality of allochthonous detrital inputs and primary production.

The present study investigates changes in benthic insect communities associated with old-field succession on the surrounding watershed, relying on the work of Woodall and Wallace (1972) on the same streams as baseline data. Current velocity and substrate composition were also investigated because of their effects on distribution of benthic insects (Hynes 1970).

Study Site

Grady Branch (Watershed 18) and Sawmill Branch (Watershed 6) are located at the Coweeta Hydrologic Laboratory, Macon Co., North Carolina. Both are first-order streams ($\sim$ 0.5−1.5 m in width) with mean depth about 15 cm. Substrate consists of pebbles and cobble (16 to <256 mm in diameter) and sand. Granitic boulder outcrops are prevalent on Sawmill Branch. Grady Branch drains an undisturbed hardwood watershed of 12.46 ha (1 ha = 10 000 m^2), with a slope of 52%. Riparian vegetation consists of chestnut oak, red maple, northern red oak, tulip poplar, pignut hickory, and black oak, with a dense understory of rhododendron. Sawmill Branch drains a watershed of 8.86 ha with a slope of 54%. This watershed was subjected to several perturbations from 1958 to 1968 including a clear-cutting, herbicide treatment, conversion to grass, and heavy fertilization (for a more complete description of watershed disturbances, see Webster and Patten 1979). Since 1968 this watershed has undergone natural secondary succession (cf. Fig 1a and b). Dominant riparian vegetation now includes various herbaceous plant species, black locust, and blackberry. Black locust is currently the dominant tree species with some specimens over 6 m in height. Sapling tulip poplar and oak are now apparent. Some grass species are still present along the stream banks.

Mean annual discharge measured by a V-notch weir is 5.64 L/s for Grady Branch and 3.38 L/s for Sawmill Branch. Water chemistry data (from Swank and Douglass 1975) are summarized in Table 1.

Materials and Methods

Each stream was sampled monthly for 1 yr beginning in November, 1977. Each month, six random 0.093 m^2 Surber samples (mesh size = 250 μm) were taken from six randomly selected sites on each stream. Samples were preserved in the field with 6% formalin containing Phloxine B dye to facilitate sorting (Mason and Yevich 1967). Current velocity measurements were made using a Gessner bag meter (Gessner 1950). The percentage of substrate was estimated visually for each sample and recorded in the field using Cummins' (1962) particle-size categories. These estimates were used to assign each sample to one of four substrate classifications: rock face (boulder or granite outcrop), cobble riffle, pebble−gravel riffle, and sandy reach.

In the laboratory, each sample was washed in a series of sieves, organic matter was decanted from inorganic matter, and insects were sorted at 7× magnification with a binocular microscope. Detritus was grouped into four categories: aquatic moss, leaf, wood and twig, and fine particulate detritus. Detritus dry weights were obtained after drying in an oven at 60°C for 24 h.

The raw data were transformed into normal equivalent scores so parametric statistical tests could be used (Thorndike 1925). A series of two-way analyses of variance (ANOVA) (Gill 1978), using stream, season, and substrate as control variables, were done to investigate insect distributions.

TABLE 1. Average annual concentrations (mg/L) of ions in Grady Branch and Sawmill Branch.[a]

Stream	NO_3-N	NH_4-N	PO_4-P	Cl	K	Na	Ca	Mg	SO_4
Grady Branch	0.003	0.004	0.002	0.538	0.461	0.883	0.626	0.296	0.298
Sawmill Branch	0.672	0.005	0.002	1.296	0.602	1.102	1.107	0.649	0.359

[a]Data from Swank and Douglass (1975).

TABLE 2. Summary of substrate type,[a] detritus standing crop (g dry wt/m^2), and current velocity (cm/s) for Sawmill Branch and Grady Branch.

	Sawmill Branch				Grady Branch			
	R.F.	C.R.	P.G.R.	S.R.	R.F.	C.R.	P.G.R.	S.R.
Number of samples	17	28	20	6	7	28	28	9
Aquatic moss	170.4	0	0	0	53.9	0	0	0
Leaf detritus	0	7.2	8.1	1.7	0	35.2	13.4	12.5
Wood and twig detritus	0	25.3	42.9	23.5	0.7	51.9	24.7	90.3
Fine detritus	0	57.4	65.5	64.8	0	54.3	31.7	36.4
Current velocity	60.03	53.05	47.62	40.08	63.67	57.74	46.2	39.0

[a]C.R. = cobble riffle; P.G.R. = pebbie−gravel riffle; S.R. = sandy reach; and R.F. = rock face. Substrate size-classes: boulder >256 mm; cobble = 64−256 mm; pebble = 16−63 mm; gravel = 2−15 mm; and sand =<2 mm.

Although a number of genera show significant differences between substrate types, these should be interpreted with caution, because detritus abundance varied significantly between substrates. No attempt was made to separate detritus and substrate effects. Therefore, some differences in substrate preference in the following discussion are undoubtedly due both to detritus abundance and/or substrate.

The data are summarized in Table 2 for substrate type, current velocity, and detritus. Because of the random sampling scheme, the number of samples of each substrate type reflects the relative abundances of the substrate types in the streams.

Results and Discussion

SUBSTRATE TYPE, CURRENT VELOCITY, DETRITUS

The random sampling method indicates that rock-face habitats are much more abundant in Sawmill Branch, but the remaining substrate types are similar for each stream.

Current velocity was highest in the rock-face habitats (Table 2), followed by cobble riffles, pebble−gravel riffles, and sandy reach habitats. There was no significant difference ($\alpha = 0.05$) in current velocity for similar substrate types between the streams.

Moss was restricted to the rock-face habitat in both streams, and standing crops (g dry wt/m^2) (Table 2) were 3 times higher on Sawmill Branch than on Grady Branch. High moss standing crops on Sawmill Branch may be due to: (1) potentially higher solar radiation on the large granite outcrop and in this stream, and (2) higher nutrient levels in Sawmill Branch (Table 1). Higher leaf and wood detritus in Grady Branch is attributed to the dense canopy of deciduous trees. Leaf detritus in Sawmill Branch consisted of grass, herbaceous plant leaves, and deciduous leaf detritus. There was no significant difference ($\alpha = 0.05$) in the standing crop of fine detritus between the two streams.

Although leaf detritus standing crops were higher on Grady Branch, the food quality of leaf detritus must be considered. Black locust, the dominant riparian species along Sawmill Branch is a legume and fixes nitrogen by means of bacteria. High nitrogen levels in leaf litter of black locust (Harlow and Harrar 1958) may result in improved detritus food quality in Sawmill Branch. Iversen (1974) found that higher gross growth efficiency in *Sericostoma personatum* Spence (Trichoptera: Sericostomatidae) was positively correlated with nitrogen content of detritus. Growth of heterotrophic microorganisms may also be enhanced by the 200× higher NO_3-N content of stream water in Sawmill Branch (Table 1). Concentrations of NO_3-N in Sawmill Branch were even higher than those reported in Table 1 during the November 1977, to October 1978, period of this study (W. Swank, Coweeta Hydrologic Laboratory, Franklin, N.C., personal communication and Coweeta Data Files). Fungi are known to extract soluble N compounds from stream water (Willoughby and Redhead 1973; Kaushik and Hynes 1971), and it has been shown that N and P additions accelerate fungal growth (Bärlocher and Kendrick 1973a). The above factors may potentially increase detritus food quality on Sawmill Branch and enhance larval growth (Cummins and Klug 1979).

Ephemeroptera — Baetis spp. (Baetidae) and *Ephemerella* spp. (Ephemerellidae) were significantly more abundant in Sawmill Branch than Grady Branch (Table 3). Both genera had significantly higher densities in the rock-face habitat. These genera are mainly fine particle feeders which seasonally ingest algae (Brown 1961; Chapman and Demory 1963; Gilpin and Brusven 1970; Woodall 1972). However, Jones (1949) reported that members of these genera may ingest large quantities of aquatic moss, which may account for higher densities of *Baetis* and *Ephemerella* in Sawmill Branch.

Paraleptophlebia sp. (Leptophlebiidae) was most common

TABLE 3. Results of ANOVA for dominant taxa showing stream and substrate preference.

	Stream[a]	Substrate[b]
Ephemeroptera		
Ephemerella spp.	S.B.	R.F.
Baetis spp.	S.B.	R.F.
Paraleptophlebia sp.	G.B.	C.R. and P.G.R.
Stenonema sp..	**	C.R.
Epeorus sp.	**	C.R.
Plecoptera		
Peltoperla maria	G.B.	**
Leuctra spp.	G.B.	C.R.
Alloperla sp.	G.B.	P.G.R. and S.R.
Nemoura	S.B.	**
Odonata		
Lanthus parvulus	S.B.	C.R. and P.G.R.
Cordulegaster erroneus	S.B.	S.R.
Trichoptera		
Parapsyche cardis	S.B.	R.F.
Diplectrona modesta	**	**
Dolophilodes distinctus	G.B.	C.R.
Rhyacophila spp.	G.B.	**
Hydroptila sp.	S.B.	R.F.
Neophylax	S.B.	**
Coleoptera		
Ectopria nervosa	**	**
Anchytarus bicolor	S.B.	**
Elmidae (larvae)	S.B.	R.F.
Elmidae (adults)	**	**
Diptera		
Nymphomyiidae	G.B.	**
Tipula spp.	S.B.	C.R.
Dicranota sp.	G.B.	**
Hexatoma sp.	G.B.	P.G.R. and S.R.
Pericoma sp.	S.B.	R.F.
Ceratopogonidae	G.B.	**
Simuliidae	S.B.	**
Chironomidae	**	R.F.

[a]S.B., significantly higher densities on Sawmill Branch ($\alpha = 0.05$); G.B.; significantly higher densities on Grady Branch ($\alpha = 0.05$); **, densities not significantly different.

[b]C.R., cobble riffle; P.G.R., pebble–gravel riffle; S.R., sandy reach; R.F., rock face; and **, no significant substrate preference.

in cobble and pebble–gravel riffle areas and was significantly more abundant in Grady Branch (Table 3). Edmunds (1978) states that members of this genus are mainly shredders and detrital collectors, which agrees with gut content studies of Woodall (1972) on the Coweeta species. The correlation between *Paraleptophlebia* sp. densities and leaf detritus standing crop in Grady Branch was significant ($r = 0.37$, $\alpha = 0.003$). These facts suggest *Paraleptophlebia* sp. may have been more abundant on Grady Branch because of large quantities of deciduous leaf detritus.

Densities of *Stenonema* sp. and *Epeorus* sp. (Heptageniidae) were not significantly different between the two streams (Table 3). Both genera are detrital collectors (Woodall 1972; J. N. Minshall 1967), although algal food may be important (J. N. Minshall 1967; Gilpin and Brusven 1970; Woodall 1972). These genera are apparently opportunistic feeders (Cummins 1973) and are not affected by differing food sources between the two streams. Densities of both genera were higher in cobble riffle areas.

Odonata — The gomphid, *Lanthus parvulus* Selys and *Cordulegaster erroneus* Hagen (Cordulegastridae) were significantly more abundant in Sawmill Branch (Table 3). *Lanthus parvulus* was more abundant in cobble and pebble–gravel riffle areas, while *C. erroneus* was more common in the sandy reach habitat. Needham and Westfall (1955) report similar habitats for these predators.

Plecoptera — *Peltoperla maria* Needham and Smith (Peltoperlidae) and *Leuctra* spp. (Leuctridae) had significantly higher densities in Grady Branch (Table 3), and were significantly correlated with leaf detritus standing crops (*Peltoperla maria*: $r = 0.66$, $\alpha = 0.001$; *Leuctra* sp.: $r = 0.45$, $\alpha = 0.0001$). *Peltoperla maria* and *Leuctra* spp. are both leaf shredders (Harper1978) and Wallace et al. (1970) showed *P. maria* had preferences for certain leaf species. Densities of *P. maria* were not significantly different between substrate types, but *Leuctra* spp. were significantly higher in cobble riffle areas.

Alloperla sp. (Chloroperlidae) was significantly more abundant in Grady Branch (Table 3). Harper (1978) reports members of this genus are collector–gatherers and predators. The feeding habits of the Coweeta species are not known.

Nemoura sp. (Neumouridae) was significantly more abundant on Sawmill Branch and densities were not significantly different between substrate types (Table 3). Harper (1978) indicates that members of this genus are collector–gatherers or shredder–detritivores.

Trichoptera — Over 90% of the *Rhyacophila* spp. (Rhyacophilidae) in Sawmill Branch were collected from rock-face habitats. However, *Rhyacophila* in Grady Branch were more evenly distributed among all habitat types. Thus while *Rhyacophila* in rock-face habitats were significantly higher in Sawmill Branch overall stream densities were higher in Grady Branch. *Dolophilodes distinctus* (Philopotamidae) was significantly more abundant in Grady Branch (Table 3). Malas and Wallace (1977) reported *D. distinctus* is generally restricted to the bottom of rocks in areas of lower current velocity. Low densities of *D. distinctus* in Sawmill Branch may be related to large amounts of interstitial sand which may destroy potential microhabitats for the large, fine-meshed catchnet constructed by this species.

Parapsyche cardis (Hydropsychidae), *Hydroptila* sp. (Hydroptilidae), and *Neophylax* sp. (Limnephilidae) had significantly higher densities in Sawmill Branch (Table 3). This distribution is directly related to the abundance of the rock-face habitat for *P. cardis* and *Hydroptila* sp., because both of these insects were largely restricted to this substrate (Table 3). Selection of the moss-covered rock face has been reported for these genera by Percival and Whitehead (1929) and Woodall and Wallace (1972). *Parapsyche cardis* was significantly correlated with moss standing crops ($r = 0.65$; $\alpha = 0.04$) and current velocity ($r = 0.24$; $\alpha = 0.04$) on Sawmill Branch, but neither correlation was significant on Grady Branch. This may be attributed to: (1) rarity of rock-face habitats in Grady Branch which were seldom sampled by

the random sampling design; and (2) the much lower standing crop of moss in Grady Branch. *Hydroptila* sp. and *Neophylax* sp. feed mainly on algae (Wiggins 1977) and their greater abundance in Sawmill Branch may be related to the more open (less dense) canopy allowing increased algal growth.

Diplectrona modesta Banks showed no significant preference for stream or habitat type (Table 3), although densities were much higher in moss-covered rock faces in Sawmill Branch, *Diplectrona modesta* was positively correlated with moss standing crop on Sawmill Branch ($r = 0.51$, $\alpha = 0.03$), but not with current velocity ($r = -0.03$; $\alpha = 0.80$); whereas, like *P. cardis*, *D. modesta* was not correlated with either moss standing crop or current velocity in Grady Branch. Malas and Wallace (1977) found the preferred microhabitat of *D. modesta* was the bottom of stones. Nevertheless, thick moss provides numerous microhabitats with respect to current velocity, while the interstitial sand in Sawmill Branch riffles may inhibit colonization by *D. modesta*.

Coleoptera — Densities of *Ectopria nervosa* (Psephenidae), a grazer (Pennak 1978), were not significantly different between the streams or substrate types (Table 3). *Anchytarsus bicolor* (Ptilodactylidae) had significantly higher densities in Sawmill Branch (Table 3). *Anchytarsus bicolor* is reported to feed on grass roots and leaf mold (Leech and Chandler 1963), and grasses are more abundant on stream banks and in the stream channel of Sawmill Branch.

Most Elmidae collected were *Optioservus* spp. and *Oulimnius* spp. *Promoresia* sp. was occasionally collected from moss-covered rock faces in Sawmill Branch. Adult elmid densities were not significantly different between the streams or between substrate types (Table 3). Conversely, elmid larvae were significantly more abundant in Sawmill Branch and had highest densities on the rock-face habitat. Brown (1976) reports adults and larvae of the tribe Elmini feed chiefly upon encrusted algae on solid substrates. The latter suggests that the higher larval elmid densities may be related to higher primary production in Sawmill Branch.

Diptera — Diptera which were significantly more abundant in Grady Branch include a new genus and species of Nymphomyiidae, the family Ceratopogonidae, and the tipulids *Dicranota* sp. and *Hexatoma* spp. Populations of the nymphomyiid were possibly destroyed by the earlier perturbations on Sawmill Branch, and the poor dispersal powers of this insect (Kevan and Cutten-Ali-Khan 1975) may account for the observed distribution.

The psychodid *Pericoma* sp. and Simuliidae had significantly higher densities in Sawmill Branch (Table 3). Egglishaw (1969) reports *Pericoma* sp. is restricted to moss, and although not confined to moss in this study, densities were 5 times higher in the moss-covered rock outcrops. Simuliid densities were not significantly different between substrates.

Tipula spp. (Tipulidae) were significantly more abundant in Sawmill Branch and had highest densities in cobble–riffle areas. Species within this large genus are regarded as shredders, collector–gatherers, scrapers, and engulfers (Byers 1978).

Chironomidae densities were not significantly different between the two streams. This family had significantly higher densities in the rock-face habitats, and their mean annual densities exceeded 23 000 m^{-2} in the thick moss of the rock-face habitats of Sawmill Branch. Chironomids composed over 50% of the total aquatic insect densities in each stream.

Benthos Comparison 1977–78 vs. 1968–69

There are several problems when one compares the present data with those of Woodall and Wallace (1972): (1) the Surber sampler used in the earlier study had net mesh openings about 1.8× larger than that used in the present study; and (2) the 1968–69 samples of Woodall and Wallace were not picked under a binocular microscope. Consequently, many small insects (e.g. Nymphomyiidae) and early instars were possibly overlooked in the 1968–69 study. This supposition is supported by total abundances which are higher in the present study.

To minimize these problems, the ratio of abundances in Sawmill Branch to those in Grady Branch were determined for each study and then compared. This procedure assumes densities have not changed in Grady Branch. The Wilcoxon signed rank test (Hollander and Wolfe 1973) was used to test differences in ratios. The following are significant changes which occurred in the 10-yr period.

Detritus

The standing crop (g dry wt./m^2) of total detritus (this included moss, as did Woodall and Wallace 1972) has increased significantly in Sawmill Branch since 1968–69 (Table 4). Various grass species and some herbaceous plants constituted most of the detritus in 1968–69 (Woodall and Wallace 1972). In addition to the preceding, deciduous tree leaves (black locust, tulip poplar, and oak), rhododendron leaves, and various herbaceous plant parts now comprise part of the detritus in Sawmill Branch. Changing riparian vegetation has undoubtedly affected Sawmill Branch in three ways: (1) increased food quality of allochthonous detritus; (2) increased food quantity through higher allochthonous inputs; and (3) reduced primary production associated with a denser canopy.

Ephemeroptera — *Stenonema* spp. have increased significantly in Sawmill Branch (Table 4), and were the only mayflies which showed significant change. Increased *Stenonema* spp. densities may be related to higher food quality of detritus on Sawmill Branch.

Odonata, Plecoptera, Trichoptera — *Peltoperla maria* was the only member of these three orders to change significantly (Table 4), and densities had increased significantly in Sawmill Branch in the present study. The increase is undoubtedly related to larger amounts of higher quality leaf detritus on Sawmill Branch, which is this insect's preferred food (Wallace et al. 1970).

Coleoptera — Densities of all coleopterans, *E. nervosa*, *A. bicolor*, elmid larvae and adults, were significantly lower in Sawmill Branch in the 1977–78 study than in 1968–69 (Table 4). These reductions are potentially related to lower

TABLE 4. Comparison of significantly different ($P < 0.05$) ratios (Sawmill Branch/Grady Branch) of aquatic insect densities and detritus for the years 1968–69 vs. 1977–78.

	Ratio (Sawmill Br./Grady Br.)		
	1968–69	1977–78	Relative change
Detritus (total)	0.899	1.38	+1.53
Moss[a]	n.a.[b]	3.16	
Leaf detritus	n.a.	.278	
Woody detritus	n.a.	.548	
Fine detritus	n.a.	1.54	
Stenonema spp.	0.27	0.93	+3.44
Peltoperla maria	0.035	0.50	+14.34
Ectopria nervosa	17.7	1.61	−10.99
Anchytarsus bicolor	21.4	3.5	−6.11
Elmidae adults	36.0	10.1	−3.6
Elmidae larvae	79.0	2.84	−27.8
Ceratopogonidae	3.52	.675	−5.22
Chironomidae	2.73	1.05	−2.6
Dicranota sp.	1.77	3.52	+2.77

[a]Moss was included with detritus by Woodall and Wallace (1972).
[b]n.a., data not available.

levels of primary production through shading since these groups are grazers. *Anchytarsus bicolor* density decrease may be related to the decrease in its food source, which is reported as grass roots (Leech and Chandler 1963).

Diptera — Ceratopogonidae and Chironomidae are now significantly lower on Sawmill Branch (Table 4). Generic and species analyses were not conducted; thus, conclusions cannot be drawn from these observations. The tipulid *Dicranota* sp. had significantly lower densities in the present study. This genus is carnivorous (Byers 1978) and the reasons for its decline are unknown.

Conclusions

Ward (1975) compared benthos in a Colorado creek with those results obtained in a similar study of the same creek 29 years earlier. Ward concluded that despite differences in flow, riparian vegetation, and temperature, macroinvertebrate composition was similar to that of the study conducted 29 years earlier. Our study differs from that of Ward's in several respects: (1) Based on Fig. 1 in Ward's (1975) study, both the magnitude and rate of changes in riparian vegetation are much greater in the southern Appalachians; (2) the stream studied by Ward is much larger, with an average discharge about 3000× that of Sawmill Branch; and (3) small woodland streams are more closely linked to terrestrial environments (Cummins 1974; Hynes 1975; Vannote et al. 1980), and one would expect such streams to be much more influenced by terrestrial succession than larger streams.

Hynes (1975) concluded that the stream is ruled by its valley. Surrounding forests exert very important influences on headwater stream ecosystems. High allochthonous organic inputs and lower solar radiation reaching the stream are two of the most obvious of these influences. The terrestrial ecosystem also influences stability characteristics of stream ecosystems. Headwater streams of forested regions have been characterized as having low resistance to perturbation and high resilience following perturbation (Webster et al. 1975; O'Neill et al. 1975; Webster and Patten 1979). However, recent studies suggest that recovery of small southern Appalachian streams from watershed disturbance is controlled by the rate of recovery of the surrounding terrestrial vegetation (Gurtz et al. 1980). That is, recovery is related to the restoration of the quality, quantity and timing of allochthonous organic inputs. Likewise, this study suggests that recovery of stream benthos trophic structure and function is related to the recovery of surrounding vegetation. Although we have documented significant changes in certain benthic taxa on Sawmill Branch over a 10-yr period, there are still significant differences between this disturbed watershed and an adjacent hardwood stream. Some of the differences between these streams can be attributed to differences in substrate availability, e.g. rock-face habitat. Major differences on Sawmill Branch since 1968 are manifested through both reduced abundances of grazer organisms and increased abundances of dominant shredders such as *Peltoperla*. This suggests: (1) terrestrial successional vegetation influences sequential changes in stream macrobenthos; and (2) restoration of the stream benthos community with respect to trophic structure and function is a long-term process dependent on recovery of terrestrial vegetation.

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Northern Squawfish (*Ptychocheilus oregonensis*) Predation on Juvenile Salmonids in Sections of the Willamette River Basin, Oregon

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BUCHANAN, D. V., R. M. HOOTON, AND J. R. MORING. 1981. Northern squawfish (*Ptychocheilus oregonensis*) predation on juvenile salmonids in sections of the Willamette River basin, Oregon. Can. J. Fish. Aquat. Sci. 38: 360–364.

Predation by northern squawfish (*Ptychocheilus oregonensis*) on salmonids was studied in spring months in several lower, free-flowing areas of the Willamette River drainage, Oregon. During 1976 and 1977, 1127 northern squawfish were collected by electrofishing and seining, and examined for food contents. Almost 59% of the squawfish stomachs contained food items, but only 2% contained salmonids. Principal foods were insects, crayfish, and sculpins (Cottidae), and the majority (over 90%) of squawfish had not consumed more than one principal food type. We suggest that previous reports of squawfish predation in flowing rivers were misleading because they were often based on artificial situations (below dams or in waters following hatchery releases) which could have inflated salmonid predation values. Further, the true predatory nature of squawfish is clouded because of their rapid digestion of food and the long lag time in examination of gut contents by previous researchers. Our sampling techniques avoided these shortcomings by sampling in free-flowing areas and by examining squawfish stomach contents immediately after capture.

Key words: squawfish, *Ptychocheilus oregonensis;* predation, food habits, Oregon

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L'article qui suit décrit les résultats d'une étude sur la prédation de la sauvagesse du nord (*Ptychocheilus oregonensis*) sur les salmonidés, au printemps, dans plusieurs basses régions, coulant librement, du bassin de la rivière Willamette en Orégon. En 1976 et 1977, nous avons recueilli par électropêche et sennage 1127 sauvagesses du nord et examiné leurs contenus stomacaux. Presque 59% des estomacs contenaient de la nourriture, mais seulement 2% de salmonidés. Les principaux organismes sont des insectes, des écrevisses et des chabots (Cottidae). La majorité (plus de 90%) des sujets n'avaient pas consommé plus d'un type principal de nourriture. Nous sommes d'avis que les rapports antérieurs sur la prédation de cette espèce dans les rivières coulant librement sont trompeurs, parce que souvent fondés sur des situations artificielles (en aval de barrages, ou après mise en liberté par une écloserie) qui auraient pu donner plus d'importance aux salmonidés comme proies de la sauvagesse. En outre, la véritable nature prédatrice de cette dernière est obscurcie du fait que ce poisson digère rapidement sa nourriture et que, dans des études menées par d'autres chercheurs, il y avait décalage entre la capture et l'examen des contenus stomacaux. Nous avons contourné la difficulté en échantillonnant des régions où l'eau coulait librement et en examinant les contenus stomacaux immédiatement après la capture.

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ACCOUNTS of northern squawfish (*Ptychocheilus oregonensis*) predation on juvenile anadromous salmonids in nursery lakes have been well documented (Foerster and Ricker 1941; Ricker 1941; Thompson and Tufts 1967). Sims et al. (1977) noted that squawfish were a major predator on migrating juvenile salmonids immediately below Columbia River dams, and Thompson (1959) found evidence of squawfish predation on salmonids in the lower sections of the Columbia River from Astoria to McNary Dam. However, all these studies used gill nets to capture squawfish, and the stomachs were not analyzed for 24 to even 72 h after capture. More recent work by Steigenberger and Larkin (1974) indicates that squawfish can clear their digestive tract in 24 h. They suggest that studies using gill nets may underestimate the extent of salmonid predation by squawfish.

A further shortcoming of previous research on predation by squawfish in rivers has been the location of sampling. Previous studies were conducted in artificial situations, such as below dams or in waters following hatchery releases of salmonids. A clear indication of the extent of squawfish predation on salmonids in free-flowing waters has yet to be undertaken.

To provide such an indication of the extent and implications of squawfish predation, a study was conducted during peak emigrations of salmonids in lower stretches of the Willamette basin of Oregon. Squawfish were captured in the spring of 1976 and 1977, and stomach contents of all squawfish were examined immediately after capture to eliminate possible bias due to digestion. Sampling was conducted in free-flowing areas of rivers, away from dams, and considerable distance below hatchery releases, to eliminate bias related to unnatural situations.

Methods — Northern squawfish and several species of salmonids were captured in three study locations in the Willamette basin in 1976: (1) the Willamette River between Buena Vista and Independence (18 km); (2) the Santiam River between Interstate Highway 5 and the confluence with the Willamette River (10 km); and (3) the lower McKenzie River from Armitage Park to the confluence with the Willamette River (7 km) (Fig. 1). In 1977, the Santiam section was extended upstream to Jefferson (15 km total), and the McKenzie section was extended upstream to Hayden Bridge (24 km total). The Willamette River was not sampled in 1977 because of logistical constraints.

The study sections were sampled by seining and drift boat electrofishing. Seines were 30.5 m × 2.5 m, with 1-cm^2 mesh. The boat was equipped with a 2500-W generator and a Coffelt model 2C VVP electrofisher with probes. The Coffelt unit was operated with pulsed direct current at 600 V. A longhandled dip net with 1-cm^2 mesh was used to retrieve stunned fish. Seines were used to sample the deeper pools, while the electrofishing gear sampled riffles and shoreline areas less than 2 m deep during straight drifts.

Sampling began in April and continued through June, coinciding with the usual emigration of several species of anadromous salmonids (*Oncorhynchus* spp., *Salmo gairdneri*). In 1976, each section was sampled three times per week. In 1977, the Santiam River section was sampled three times weekly while the McKenzie River section was sampled twice

FIG. 1. Location of sampling sites for northern squawfish in the Willamette River basin, 1976 and 1977; (1) Willamette River, (2) Santiam River, (3) lower McKenzie River. Release sites indicated are those of hatchery-reared anadromous salmonids.

weekly. On each sampling day, a standardized effort of 2 h of electrofishing and six seine hauls was made. Sampling usually began between 06:00 and 07:00, and was completed 6 h later.

Numbers of squawfish and migratory or resident salmonids in each study section were not estimated. Substantial wild populations of resident trout and anadromous salmonids are found near our sampling sites. Also, substantial numbers of anadromous hatchery salmonids were released upstream of the study sections during this period (Table 1). We assumed that many of these hatchery fish were migrating through the study sections during the sampling periods. More than 4.7 million juvenile salmonids was released above the Santiam study section in 1976, while only 1.45 million was released in 1977. Conversely, 0.62 million hatchery fish was released above the McKenzie section in 1976, and 1.0 million was released in 1977. Most of these releases were made in the upper, fast-flowing sections of the Willamette tributaries, including areas near Foster Dam and Waterloo on the South Santiam, Minto Dam, and Stayton on the North Santiam, and Leaburg Dam on the McKenzie (Fig. 1). We sampled each of these upper release areas with electrofishing gear for 4 d in June to compare relative abundance of squawfish in the

TABLE 1. Anadromous hatchery salmonids released in April and May 1976 and 1977 in the Willamette River basin upstream of the squawfish study sections. Sizes of smolts released range from 8 to 25 cm FL.

		Number of smolts released				
River	Year	Steelhead (*Salmo gairdneri*)	Fall chinook salmon (*Oncorhynchus tshawytscha*)	Spring chinook salmon (*O. tshawytscha*)	Coho salmon (*O. kisutch*)	Total
McKenzie	1976	150 000	0	400 000	70 000	620 000
Santiam	1976	450 000	2 270 000	1 950 000	50 000	4 720 000
Willamette	1976	600 000[a]	5 320 000[a]	6 620 000[a]	280 000	12 820 000
McKenzie	1977	130 000	0	900 000	0	1 030 000
Santiam	1977	400 000	190 000	860 000	0	1 450 000

[a]Smolts released in the McKenzie and Santiam systems were included in the Willamette group since these fish could also have emigrated past this lower study section.

hatchery-release areas and in our lower-river study sections.

An unexpected release of 11 700 steelhead trout, *Salmo gairdneri* (avg 20 cm fork length (FL)), was made into our Santiam study section on April 16, 1976. Normally hatchery releases were made 30 km upstream from our study areas. Because these fish were identifiably marked, they provided an opportunity to determine predation results following an introduction of salmonids in an area populated with squawfish.

All captured squawfish were processed immediately, and length and stomach contents were recorded. Slightly greater numbers of females were captured (48% versus 31% males and 21% immature), but we have no information to show that sex of squawfish may have been a significant factor in predation. Frequency of occurrence of food items is expressed as the number (or percentage) of squawfish stomachs that contained a given food item.

Results — We found that squawfish predation in natural free-flowing rivers during peak emigration of anadromous salmonids was not as great as that reported in lakes or immediately below a dam near a hatchery-release site. Stomachs of 1127 squawfish were examined immediately after capture, and 59% contained food items (Table 2). A total of 17% of the stomachs in 1976 and 25% of the stomachs in 1977 contained fish or fish parts. However, most of those fish were not salmonids, but were primarily sculpins (Cottidae). Only 3% of the stomachs in 1976 and less than 1% of the stomachs in 1977 contained salmonids. This pattern of food consumption was similar for all study sections. We separated the food of northern squawfish into four major categories and found that only 5 and 6% of the squawfish sampled in 1976 and 1977, respectively, contained more than one kind of major food item. Similarly, Thompson (1959) noted that multiple food items were present in only 3% of the squawfish containing food. Apparently, squawfish are opportunistic and will concentrate specifically on the food source that is most abundant or easiest to capture. This opportunistic behavior may explain why squawfish will become such active predators on salmonids immediately after a hatchery release or below a dam, where unnaturally high concentrations and disorientation can occur, and weak and stunned fish are present.

The unexpected release of hatchery steelhead smolts in the lower Santiam River sampling area may have increased the percentage of salmonids found in squawfish stomachs in 1976. Eighteen salmonids were identified in squawfish stomachs, of which 7 (39%) were steelhead smolts from this release.

Fork lengths of squawfish ranged from 8 to 55 cm, averaging 30 cm, but the range of squawfish that had eaten salmonids was from 16.5 to 48 cm. Twenty-five percent of the squawfish that had eaten salmonids were less than 30 cm in length, while the remaining 75% were greater than 30 cm. We estimate that more than 90% of the squawfish examined were of sufficient size that they could have been potential predators on juvenile salmonids.

Relative to salmonids, fewer squawfish were caught each year on the McKenzie River than on the Santiam or Willamette rivers (Table 3). When related to length of stream sampled, the incidence of squawfish per kilometre is highest in the McKenzie River in 1976, (39 squawfish/km), but lowest there in 1977 (7/km), when the sampling section was extended upstream (compared to 3/km in the Willamette in 1976; and 23/km in the Santiam in 1976, and 27/km in 1977). Yet sole reliance on incidence per kilometre values may not completely categorize the stream populations. Effort (seine hauls and electrofishing time) was constant each year. When only squawfish and salmonids are considered, 4% of the fish captured in 1976 from the lower McKenzie River were squawfish, while 13 and 9% of the fish from the lower Santiam and Willamette rivers, respectively, were squawfish. The relative abundance of squawfish and salmonids were similar in 1977: only 3% of the fish collected in the lower McKenzie River were squawfish, while 11% of the fish sampled in the lower Santiam River were squawfish. These results only pertain to salmonids and squawfish greater than or equal to 7 cm in length because the mesh size of the seine and dip net was too large to capture consistently smaller fish. Nevertheless, it implies that trout productivity is higher in the McKenzie River, possibly because of a more compatible pool–riffle ratio.

The proportion of hatchery fish captured in the lower river collection areas to numbers of anadromous salmonids released in areas upstream varied between rivers and between sites. The proportion was always higher in the McKenzie than in the Santiam River (0.19 and 0.36% versus 0.03 and 0.19%, respectively), and the proportion was higher in 1977 than in 1976. The greater proportion obtained in 1977 is probably due to the increased length of sampling areas on both streams

TABLE 2. Stomach contents of northern squawfish captured in the Willamette River system in spring 1976 and 1977.

Food items[a]	1976		1977		Total	
	N	%[b]	*N*	%[b]	*N*	%[b]
Squawfish examined	552		575		1127	
Stomachs with food	328	59.4	334	58.1	662	58.7
A. Fish						
1. Salmonids		3.3		0.7		2.0
a) Steelhead trout	8	1.5	0	0.0	8	0.7
b) Trout	0	0.0	2	0.4	2	0.2
c) Salmon	9	1.6	2	0.4	11	0.9
d) Unknown	1	0.2	0	0.0	1	0.1
Subtotal	18		4		22	
2. Sculpins	64	11.6	98	17.0	162	14.4
3. Other fish[c]	15	2.7	40	7.0	55	4.9
Total fishes	97	17.6	142	24.7	239	21.2
B. Insects	128	23.2	77	13.4	205	18.2
C. Crayfish	69	12.5	104	18.1	173	15.4
D. Other food[d]	54	9.8	30	5.2	84	7.5

[a]Four major food types are listed; some squawfish stomachs contained more than one food type so that subtotaled percentages can be greater than 100%.

[b]Percentage occurrence based on the total number of squawfish examined, and the total number that contained food items.

[c]Cyprinidae, Catostomidae, and Petromyzontidae.

[d]Algae, fish eggs, earthworms, berries, and leaves.

TABLE 3. Number of northern squawfish and migrating and residual salmonids collected in study sections of lower McKenzie, Santiam, and Willamette rivers in spring 1976 and 1977.

Fish	1976			1977	
	McKenzie	Santiam	Willamette	McKenzie	Santiam
Squawfish	52	230	270	165	410
Salmonids:[a,b]	1449	1575	2924	4846	3320
Hatchery steelhead	60	463	139	224	428
Wild steelhead	5	118	14	65	207
Resident rainbow trout	50	7	3	332	158
Resident cutthroat trout	174	154	23	588	134
Hatchery spring chinook	196	51	245	317	219
Wild spring chinook	27	30	256	93	43
Fall chinook salmon	937	721	2244	3187	2115
Coho salmon	0	31	0	40	16

[a]The separation between spring and fall chinook salmon is arbitrary and was done by size. Chinook less than 10 cm were considered fall chinook while fish greater than 10 cm were separated into hatchery or wild spring chinook by degree of fin erosion.

[b]The separation between hatchery and wild steelhead smolts was done by inspection of fins. Deformed fins, particularly dorsal and caudal, are usually evident in hatchery raceway reared fish. Steelhead smolts were distinguished from resident rainbow trout by the slimmer shape, silvery coloration, darkened snout, and absence of parr marks in steelhead smolts.

(50% longer stretch on the Santiam and 243% longer stretch on the McKenzie the 2nd yr) and a greater effort (two rivers sampled in 1977 instead of three). Similarly, the higher proportion of salmonids captured in the McKenzie may also be a function of length of sampling area and sampling efficiency. The essential aspect of sampling is that squawfish were captured along with salmonids, so that potential predators were found in waters also containing migrating salmonids.

Numbers of squawfish and wild rainbow (*S. gairdneri*) or cutthroat trout (*S. clarki*) caught per sampling day in 1976 and 1977 fluctuated between the study rivers. Catches averaged 6.0, 11.8, and 12.9 squawfish and 30.2, 7.9, and 1.1 resident trout per day on the McKenzie, Santiam, Willamette sampling sites, respectively. The role of resident trout as predators on small salmonids was not studied, but the potential could be higher in the McKenzie River where trout populations were higher (trout: squawfish ratios 4.3−5.6:1 in the McKenzie,

0.7:1 in the Santiam, 0.1:1 in the Willamette).

Discussion — Squawfish predation on salmonids in the investigated sections of the Willamette River was found to be less than that reported in other areas. Thompson and Tufts (1967) found that 32% of squawfish sampled with gill nets from Lake Wenatchee, Washington, contained hatchery-released sockeye salmon (*Oncorhynchus nerka*). Similarly, Sims et al. (1977) found that 21% of gill net captured squawfish, immediately below lower Granite Dam on the Columbia River, contained salmonid remains. Only 2% of the stomachs of the 1127 squawfish that we captured during the salmonid emigration months of April, May, and June contained any salmonids. Our percentage may be inflated by the steelhead found in the stomachs of squawfish captured in the localized area of a hatchery release. Deletion of these fish would reduce the overall percentage to 1.4%.

Thompson (1959) examined the stomachs of 3546 squawfish captured with gill nets in the lower free-flowing Columbia River from McNary Dam to Astoria and found 7.5% of the squawfish contained salmonids. Salmon and sculpins comprised 87 and 6%, respectively, of all fish found in those squawfish stomachs. In our study, salmonids and sculpins comprised 10 and 68%, respectively, of all fish found in squawfish stomachs. Though Thompson's study covered a large area in the lower Columbia River, and included squawfish collected over a calendar year, some of those samples were collected in areas immediately below five fish hatcheries during periods of hatchery releases. Although those results established the extent of losses of salmon to squawfish, we believe they should not be considered representative of the total river salmonid–squawfish situation.

Ricker (1941) used gill nets to capture squawfish and found that 81% of the sampled squawfish had empty stomachs. Thompson (1959) examined squawfish taken by similar gear and found 63% empty stomachs. We inspected squawfish immediately after capture by seines and electrofishing and detected only 41% empty stomachs. However, the other studies included squawfish collected during winter months, a time when incidence of empty stomachs is greatest. Thus, the higher rates of empty stomachs are not unexpected.

Steigenberger and Larkin (1974) found squawfish have a digestion rate of 14%/h, and most fish clear their stomach contents in 24 h. Steigenberger and Larkin force-fed squawfish to determine digestion rates, so the normal evacuation rate may be even more rapid, as force-feeding has been shown to slow digestion in some fishes, such as walleye, *Stizostedion vitreum vitreum* (Swenson and Smith 1973). These results suggest that collections using gill nets that were not checked for 24–72 h after setting may have underestimated the food abundance in squawfish. By using seines and electrofishing gear we could more rapidly examine stomach contents before digestive processes could influence the results. Consequently, we found a higher percentage of stomachs containing food items.

Public agencies have long used chemicals to reduce or eradicate squawfish, but the long-term effects of this type of treatment may actually increase squawfish predation on salmonids in free-flowing rivers. If squawfish and their more abundant natural foods (e.g. sculpins, crayfish, aquatic insects) are destroyed in an area, other squawfish may soon recolonize the treated area (Reid 1971). However, certain preferred food items, such as sculpins, are slow recolonizers (Bond 1963). As a result, predation on hatchery-released salmonids could be higher in the years following treatment, and competition between squawfish and salmonids for existing food items could be increased. Further, we found overall squawfish predation to be quite low in lower sections of free-flowing systems, and piscicide treatment in these areas may not be economically or biologically justified to improve the survival of anadromous or resident salmonid populations.

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Drug Immobilization of Walrus (*Odobenus rosmarus*)

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Five out of nine walrus (*Odobenus rosmarus*) were successfully immobilized at Round Island, Alaska, in May of 1978 by combinations of phencyclidine hydrochloride and acepromazine hydrochloride. A crossbow was an effective delivery technique. Walruses that had recently hauled out were more suitable for immobilization than well-rested animals. Care was taken to prevent walruses from overheating or suffocating.

Key words: walrus, *Odobenus rosmarus;* immobilization, marine mammal

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Grâce à des combinaisons d'hydrochlorure de phencyclidine et d'hydrochlorure d'acépromazine, on a pu immobiliser avec succès cinq sur neuf morses (*Odobenus rosmarus*) à l'île Round en Alaska en mai 1978. L'arbalète a été un instrument efficace de lancement. Il était préférable d'immobiliser des morses qui avaient atterri récemment plutôt que des animaux bien reposés. On s'est efforcé de prévenir l'échauffement ou la suffocation des animaux.

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IN this note the results of drug immobilization experiments on free-living walruses are reported. Immobilization may be necessary in future studies as walruses are too large and powerful to be restrained physically under field conditions.

From May 13 to May 21, 1978, we attempted to immobilize nine Pacific walruses (*Odobenus rosmarus divergens*) hauled out at Round Island, Alaska (58°36′N, 159°58′W, see Miller 1976 for description of island habitat). We used combinations of phencyclidine hydrochloride (Sernylan) and acepromazine hydrochloride (Lentfer 1969; Seal et al. 1970; Hofman 1975) in eight attempts, and one attempt was made with ketamine hydrochloride (Williams and Kocher 1978; Hunter et al. 1977). Our target dose range of 0.2–0.3 mg/kg of Sernylan was based on previous immobilization experiences with Weddell seals (Hofman 1975) and walruses (F. H. Fay, University of Alaska, Fairbanks, Alaska, personal communication). Initially we used a capture rifle (Lentfer 1969); however, the report of the rifle disturbed the walruses. In the last six immobilization attempts we used a crossbow to deliver the drugging darts. (Darts were attached to the arrow with Palmer Chemical Company arrow adapters.) Animals were approached within 25 m. Care was taken to be quiet and to remain out of sight during the approach. The occurrence of large boulders on the beach made the approach considerably easier. Only animals that were considered to be sleeping were used as subjects. Animals were darted either in the rear flank or in the front shoulder with a 3.5 in-barbed needle. There were no indications that the capture dart ever failed to fire or that the needles were too short to inject the drugs directly into muscle tissue.

Typically, the animal's reaction to the dart involved rearing the head back and looking around. As long as the rest of the herd was calm, the darted animal would not move into the water. In the three cases where the capture rifle was used, instead of the crossbow, the animal's reaction was to move

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TABLE 1. Summary of data related to immobilization of walrus on Round Island, Alaska.

Animal no.	Drug	Dose (mg)	Estimated weight (kg)	Estimated dose/weight (mg/kg)	Latent period (min)	Effect
1	Ketamine	900	1100	0.8	—	After 47 min animal went in water. Ineffective dose.
2	Sernalyn	250	1100	0.2	22	Animal lightly sedated. After 56 min animal mobile (went in water).
3	Sernalyn Acepromazine	375 10	1700	0.2	—	Animal immediately went in water.
4	Sernalyn Acepromazine	350 15	1600	0.2	35	Animal moderately sedated. After 1 h 59 min animal awkwardly mobile.
5	Sernalyn Acepromazine	325 15	1600	0.2	40	Animal lightly sedated, able to move head while being worked. After 1 h 45 min animal mobile.
6	Sernalyn Acepromazine	340 15	1600	0.2	33	Animal moved down beach, before becoming immobile. After 50 min one convulsion occurred and the animal died.
7	Sernalyn Acepromazine	450 5	1350	0.3	20	Animal heavily sedated. After 4 h 7 min animal mobile.
8	Sernalyn Acepromazine	360 15	1100	0.3	38	Animal moderately sedated. After 4 h 23 min animal mobile.
9	Sernalyn Acepromazine	360 10	1100	0.3	26	At 33, 39, and 41 min after injection convulsions occurred. After 3rd convulsion animal died.

towards the water's edge, and often animals near the darted individual were disturbed. We noticed that animals that were at the landward edge of the herd did not move as close to the water when they were disturbed as did animals that were at the center of the herd. We also noticed, after many unsuccessful stalks, that individuals that had been hauled out for several days were very difficult to approach without disturbing them. On the other hand, animals that had recently returned to the island to haul out were quite easy to approach. This may also be confounded by the cyclic nature of hauling patterns. In general, the number of hauled out animals would cycle from thousands of animals to a few animals in roughly a 10-d period. During the buildup, the herds were large and animals were easy to approach. During the decline phase, herds were generally smaller, and animals were much more difficult to approach.

Four different combinations of drugs and dosage were tried (Table 1); ketamine (0.8 mg/kg); Sernylan (0.2 mg/kg); Sernylan (0.2 mg/kg) and acepromazine (0−15 mg); and Sernylan (0.3 mg/kg) and acepromazine (5−15 mg). The ineffective dose of ketamine was the maximum that could be delivered by a single dose, and therefore, further experiments with ketamine were discontinued.

The symptoms associated with Sernylan and Sernylan/acepromazine injections were similar to those described by Hofman (1975) for Antarctic seals. We observed an increase in nasal secretions and tear formation (first effect), resting of the head on the tusks (second effect), and the lack of response to mechanical stimulation (third effect). An animal was considered lightly sedated if it could still move its head slightly while being handled. With light sedation, eye reflexes were good and breathing was regular (8−15 breaths per minute). A moderately sedated animal had no head control, good eye reflex, and a breathing rate between five and seven breaths per minute. A heavily sedated animal had no head control, a very weak eye reflex, and a breathing rate of two to four breaths per minute. Lightly sedated animals were mobile within 2 h after immobilization; moderately sedated animals were mobile between 2 and 4 h after immobilization; and heavily sedated animals were only awkwardly mobile between 4 and 7 h after immobilization.

Thermoregulation is disrupted by Sernylan (Seal et al. 1970), and heat stress may have occurred in animals immobilized for more than several hours on warm, sunny days (daytime temperatures reached 15°C during the study period). We attempted to minimize this problem by pouring cold water over heavily sedated animals. Heavily sedated animals with weak eye reflex were also exposed to possible eye damage from flies that occur on the hauling grounds. Another potential problem is the position of the immobilized animal relative to the slope of the beach. The two fatalities that occurred involved animals that moved toward the water's edge prior to complete immobilization. This positions the head and chest lower than the abdomen. The resulting pressure of the abdominal viscera on the diaphragm may have inhibited the respiration of the drugged walruses. We feel this was not simply an overdose of the drug because both animals were mobile just prior to when their breathing stopped. Most hauled out

walruses lay facing up the incline of the beach, which may facilitate breathing.

We believe that additional immobilization experiments should be conducted. Our results indicate that a dose of 0.2–0.3 mg/kg of Sernylan is a reasonable dosage, but because of respiratory depression, alternatives to phenothiazine compounds should be considered (e.g. valium). Sernylan is not currently available and other immobilizing agents presumably must be used in the future. Based on our observations, future researchers, in developing adequate immobilization techniques for walrus, should consider the importance of delivery techniques, the sensitivity of walruses to Sernylan, and the potential problem of overheating. We recommend the crossbow as a reliable means of projecting the drugging darts, and caution against attempting immobilization research where the slope of the terrain is not level. In addition, we recommend that researchers, when choosing individuals to immobilize, select individuals that have recently hauled out and are part of a large herd.

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Use of Beef Tallow as an Energy Source in Coho Salmon (*Oncorhynchus kisutch*) Rations[1]

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YU, T. C., AND R. O. SINNHUBER. 1981. Use of beef tallow as an energy source in coho salmon (*Oncorhynchus kisutch*) rations. Can. J. Fish. Aquat. Sci. 38: 367–370.

Four experimental diets containing different proportions of beef tallow and salmon oil were prepared. Each diet was fed to triplicate groups of juvenile coho salmon (*Oncorhynchus kisutch*) for 14 wk. The final fish weight was greatest with a diet containing 8% each of tallow and salmon oil. Fatty acid analysis indicated that the dietary salmon oil provided adequate quantities of the ω3 fatty acids required by the coho salmon. Fish body lipid saturation increased only slightly as the concentration of tallow in the diet increased. Beef tallow was found to be a good source of dietary energy when used in conjunction with a fish oil rich in ω3 fatty acids.

Key words: beef tallow as source of dietary energy, coho salmon (*Oncorhynchus kisutch*), growth, feed efficiency, body lipid composition

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L'expérience décrite dans le présent article a été menée avec quatre régimes alimentaires contenant diverses proportions de suif de boeuf et d'huile de saumon. Nous avons présenté chacun de ces régimes à des groupes en triple de jeunes saumons coho (*Oncorhynchus kisutch*) pendant une période de 14 semaines. Le poids final le plus élevé des poissons a été atteint avec un régime contenant 8% chacun de suif et d'huile de saumon. L'analyse des

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acides gras indique que l'huile de saumon dans le régime fournit des quantités adéquates d'acides gras ω3 requis par les saumons coho. La saturation en lipides corporels n'augmente que légèrement avec l'augmentation de concentration de suif dans le régime. Le suif de boeuf est une bonne source d'énergie lorsqu'utilisé avec une huile de poisson riche en acide gras ω3.

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BEEF tallow is one of the most abundant and least expensive fats used extensively in the formulations of animal feeds and pet foods. A substantial saving in feed costs could be realized if tallow can be used successfully to substitute for part of the expensive and often unobtainable fish oils as a source of energy in fish feeds.

Assimilation of tallow by warm water fish had been investigated by Stickney and Andrews (1972). They reported that a fish feed containing 10% beef tallow promoted fast growth of channel catfish (*Ictalurus punctatus*) at water temperatures of 26 to 30°C.

Previously we described the incorporation of an animal fat (lard) in a diet that supported a good growth of rainbow trout, *Salmo gairdneri* (Yu et al. 1977). Beef tallow, however, is considerably more saturated than lard. The ability of cold-water fish to assimilate this high melting point fat and its influence on fish body lipid composition must be ascertained before its use can be recommended.

In this paper we compare the influence of four diets containing various concentrations of beef tallow and fish oil on growth of coho salmon, feed efficiency, and changes in the fatty acid (FA) composition of fish body lipid.

Materials and methods — Coho salmon fry hatched in the Food Toxicology and Nutrition Laboratory, Corvallis, Oregon, were fed for 4 mo a diet of casein, gelatin, dextrin, and salmon oil, as described by Lee and Putnam (1973). The fish were then randomly divided into 12 groups of 50 fish per group. Each group was stocked in a 40-L perforated plastic container. Three containers were placed in a fiber glass tank 1040-L capacity. Well water was introduced into each tank continuously at a flow rate of about 8 L/min. Water temperature was constant at 11.5°C. Oxygen content, determined weekly, was in excess of 8 mg/L in each container.

Four isocaloric and isonitrogenous diets were prepared. The composition and ingredients were the same in these diets (Table 1) except for the variation in lipids (Table 2). Salmon oil used in the diets was obtained from a commercial source in Astoria, Oregon. Tallow was prepared by rendering the beef fat obtained from a local slaughterhouse. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), 0.01% each, were added to the fats to minimize autoxidation. The methods of diet preparation were described previously (Sinnhuber et al. 1977), and the finished soft diet contained 60% moisture.

Each diet was hand-fed three times daily and 6 d each week to triplicate groups of fish placed within a tank. Food was offered as long as the fish continued active feeding. Feed consumption and fish mortality in each group were recorded daily; the fish were weighed every 2 wk. The feeding experiment was terminated at the end of 14 wk. The final fish weight gain and feed consumption were calculated.

TABLE 1. Composition of experimental diet (%) fed to coho salmon.

Casein:gelatin (3:1)	44.44
Dextrin	12.80
Lipid[a]	16.00
Cellulose mix[b]	19.46
Premix[c,d,e]	7.30
kcal/100 g[f]	350

[a]Lipid composition in each diet is presented in Table 2.
[b]Cellulose mix comprised: α-cellulose 90% + carboxymethylcellulose 10%.
[c]Premix (% in diet): mineral mix, 4.00; vitamin mix, 2.00; DL-methionine, 0.20; DL-tryptophane, 0.10; choline chloride, 1.00; total = 7.30%.
[d]Mineral mix: Bernhart–Tomarelli salt mix.
[e]Vitamin mix supplies (mg/kg of diet): thiamine, 64; riboflavin, 144; niacinamide, 512; biotin, 1.6; Ca-pantothenate, 288; pyridoxine, 48; folic acid, 19.2; menadione (water soluble), 16; cobalamine, 0.16; *i*-inositol, 2500; ascorbic acid, 1200; PABA, 400; ergocalciferol (D_2), 4000 IU/kg; vitamin A (acetate), 25 000 IU/kg; α-tocopherol acetate 660 IU/kg.
[f]Diet calorie calculated on basis of 90% protein in casein and gelatin, 90% carbohydrate in dextrin, 4.0 kcal/g protein and carbohydrate, and 9 kcal/g lipid (1 kcal = 4.1868 J).

At termination of the experiment, seven whole fish from each group were homogenized for proximate analysis, using methods described in Horwitz (1970).

Four fish from each group were taken for fatty acid determination. Total lipid was extracted from the homogenized whole fish by the method of Folch et al. (1957). Fatty acid methyl esters were prepared from the extracted lipid by the method of Morrison and Smith (1964). Gas–liquid chromatographic (GC) separation and identification of FAs were performed as described previously by Lee et al. (1967).

An analysis of variance was carried out at 5% level of significance on the values of fish weight gain and feed conversion.

Results and discussion — At termination of the feeding experiment, the stomach and intestines contents of fish from each group were examined. No trace of undigested beef tallow was found, indicating that coho salmon were able to digest and utilize the high melting point tallow when it was mixed with unsaturated salmon oil. Fish in all groups were healthy and only one fish died in one of the diet-1 triplicate groups.

The average final fish weight was greatest in the group of fish fed diet 3, containing 8% tallow and 8% salmon oil (Table 2). The percentage weight gain of diet 3 fish was also highest but not significantly different from diets 1 and 2 fish. The weight gain of diet 4 group was significantly less than other groups. Apparently too much saturated beef tallow in

TABLE 2. Effect of dietary lipids on fish weight gain and feed efficiency.[a]

Diet no.	Dietary lipid composition (%)		Average fish weight (g)		% wt. gain	Dry feed eaten (g)	Feed conversion (gain/feed)	Protein efficiency (%)
	Salmon oil	Beef tallow	Initial	Final				
1	16	0	3.1	16.8	439	647	1.05	2.65
2	12	4	3.1	16.9	447	656	1.05	2.63
3	8	8	3.3	18.2	454	713	1.04	2.61
4	4	12	3.2	15.3	383[b]	644	0.94	2.36

[a]Percentage weight gain, dry feed eaten, and feed conversion are average values of triplicate samples.
[b]Values for diet 4 are significantly different ($P < 0.05$) than diets 1, 2, or 3.

TABLE 3. Proximate analysis of coho salmon fed experimental diets for 14 wk.

Diet no.	Percentage			
	Moisture	Ash	Fat[a]	Protein
1	71.6	2.06	9.47	15.4
2	71.7	2.25	9.16	16.0
3	71.1	2.23	8.43	15.9
4	72.3	2.21	8.54	16.3

[a]Diet 1 and diet 3 only were significantly ($P < 0.05$) different.

TABLE 4. Percentage fatty acid composition of dietary lipids and coho salmon body lipids extracted from whole fish.

Fatty acids	Dietary lipids in diets				Body lipids in diets			
	1	2	3	4	1	2	3	4
14:0	4.5	4.3	4.1	3.9	3.9	3.8	3.4	3.3
14:1	0.4	0.5	0.5	0.6	0.5	0.5	0.6	0.6
15:0	0.6	0.6	0.6	0.6	0.3	0.5	0.4	0.4
16:0	13.9	17.0	20.2	23.2	13.8	14.3	15.9	16.3
16:1	6.4	5.6	4.8	4.0	8.3	8.0	8.2	8.1
17:0	0.7	0.9	1.1	1.4	0.8	0.9	1.0	0.9
17:1	1.3	1.2	1.0	0.8	1.0	1.1	1.2	1.0
18:0	2.8	8.7	14.6	20.5	3.4	4.7	6.1	7.1
18:1ω9	21.5	24.9	28.3	31.7	28.9	33.0	37.5	43.0
18:2ω6	1.7	1.5	1.2	1.7	2.8	3.3	3.3	4.1
18:3ω6	0.5	0.4	0.3	0.1	0	0	0	0
18:3ω3	1.2	1.1	0.9	0.7	0.9	1.0	0.9	0.6
20:1ω9	11.6	8.7	5.8	2.9	10.4	8.0	6.0	4.1
18:4ω3	2.0	1.5	1.0	0.5	Trace	Trace	Trace	Trace
20:2ω6	0.4	0.3	0.2	0.1	0.5	1.2	0.9	0.8
20:4ω6	0.6	0.4	0.3	0.2	0.6	0.5	0.4	0.3
22:1ω11+13	10.6	8.0	5.3	2.7	6.1	4.7	3.3	2.1
20:5ω3	7.8	5.9	3.9	2.0	4.7	3.9	2.5	1.4
22:4ω6	0.4	0.3	0.2	0.1	0.4	0.3	Trace	Trace
22:5ω6	0.9	0.7	0.4	0.2	0.4	0.5	Trace	0.3
22:5ω3	1.8	1.4	0.9	0.5	2.0	1.6	1.2	0.6
22:6ω3	8.4	6.3	4.2	2.1	10.2	8.3	7.1	5.2

this diet had impaired the diet quality and suppressed the fish growth.

Similar results were demonstrated with rainbow trout by Takeuchi et al. (1978), who used hydrogenated fish oil (77.6% saturated) and cuttlefish liver oil as sources of dietary lipid. They reported that a diet containing 4% hydrogenated fish oil and 6% cuttlefish liver oil supported a greater trout weight gain than that of the control diet containing 10% fish liver oil.

Feed efficiency and protein efficiency of diets 1, 2, and 3 were approximately equal and were significantly greater than that of diet 4 (Table 2).

No significant differences in protein, moisture, and ash were indicated (Table 3) in body composition of fish in the four diet groups. Fish fed diet 3 appeared to accumulate less body fat than did fish on diet 1 (Table 3).

The changes in concentration of individual FA in fish body lipid induced by addition of beef tallow in the diets are presented in Table 4. The concentration of 16:0, 18:0, 18:1, and 18:2 FAs increased as the level of tallow increased in the diets. The concentration of 20:1, 20:4, 22:1, 20:5, 22:5, and 22:6 FAs decreased; the concentration of 14:0, 14:1, 16:1, 17:0, and 17:1 FAs was not affected by dietary lipids. The absence of 20:3ω9 fatty acid signified that there was no essential FA deficiency (Castell et al. 1972) in any group. The ω3 FAs provided by dietary salmon oil apparently had satisfied the essential FA requirement of the fish.

The 18:1 concentration of fatty acid in fish body lipid was directly proportional to dietary saturated FA concentration and inversely proportional to dietary polyunsaturated FAs (ω3 + ω6). Our findings are in agreement with lipid metabolism in rat liver observed by Caster et al. (1966).

The influence of dietary lipids on fish body lipid composition is more evident when saturated and unsaturated fatty acids are grouped together (Table 5). Higher levels of dietary tallow reduced the total ω6 FAs from 4.5% in diet 1 to 1.8% in diet 4. However, the total ω6 FAs remained fairly constant at about 5% in the body lipid of fish from each group.

The total ω3 FAs in body lipid of fish from diet 1 and 2 groups was lower than in the respective dietary lipids, indicating that coho salmon used a part of dietary ω3 FAs for energy. When the dietary total of ω3 FAs was lowered to 10.9 and 5.8% (diets 3, 4), less ω3 FAs were used up and the percentage of total ω3 FAs in fish body lipid was higher than the ω3 FAs of their respective dietary lipids. It appears that fish are able to conserve important FAs when supplies are low.

The total monoenoic acids in dietary lipids were successively lowered from diets 1 to 4, and yet the monoenoic acid concentration in body lipid was highest in diet 4 fish and lowest in diet 1 fish. A part of the monoenoic lipid may have been synthesized by the fish.

In dietary lipids, the concentration of total saturated FAs increased markedly from 22.5% in diet 1 to 40.6% in diet 4. The total saturated FAs in fish body lipids increased only moderately from 22.2 to 28.0% (diet 1 and 4 fish). Much of the dietary saturated FAs had been used up. The degree of

TABLE 5. Effect of dietary lipids on fatty acid composition of fish body lipid. Values are the percentage of total fatty acids in lipids.

	Diet 1		Diet 2		Diet 3		Diet 4	
Classes of fatty acids	Dietary lipid	Body lipid	Dietary lipid	Body lipid	Dietary lipid	Body lipid	Dietary lipid	Body lipid
ω3	21.2	17.8	16.1	14.8	10.9	11.7	5.8	7.8
ω6	4.5	4.7	3.6	5.8	2.8	4.6	1.8	5.5
Monoene	51.8	55.2	48.7	55.3	45.7	56.8	42.7	58.9
Saturated	22.5	22.2	31.6	24.2	40.6	26.8	49.6	28.0

saturation or unsaturation of fish body lipid was reported to be influenced by environmental temperature (Knipprath and Mead 1968). In our experiment, when dietary saturated tallow increased, the fish responded by depleting more saturated and conserving more unsaturated FAs to maintain a desirable body lipid unsaturation under the low water temperature environment.

We have demonstrated that beef tallow is a good source of dietary energy for fish when used in conjunction with a fish oil. Coho salmon fed a test diet containing 8% each of tallow and salmon oil attained the greatest final fish weight. The fish weight increased about sixfold in 14 wk. Feed efficiency approached one unit weight of dry feed to produce one unit weight of wet fish. Only a slight increase in fish body lipid saturation was observed.

The market price of beef tallow is considerably lower than fish oil. By using 8% tallow and 8% fish oil instead of 16% fish oil in a fish feed, a significant saving in feed costs could be realized.

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Estimation of Potential Catches of an Unexploited Stock of Soft-Shell Clam (*Mya arenaria*) from Length Composition Data

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We describe a technique, derived from the von Bertalanffy growth curve and from the mortality equation, which allows the estimation of potential catches from an unexploited stock of mollusca on the basis of the population's length composition data. Comparison with results obtained from age composition data shows that both methods give similar values for mortality rates and yields. That technique could be very useful when it is difficult or impossible to obtain systematic age readings.

Key words: Mya arenaria, growth, mortality, yield

BRÊTHES, J.-C. F., AND G. DESROSIERS. 1981. Estimation of potential catches of an unexploited stock of soft-shell clam (*Mya arenaria*) from length composition data. Can. J. Fish. Aquat. Sci. 38: 371–374.

Les auteurs décrivent une technique dérivée de l'équation de croissance de von Bertalanffy et de l'équation de mortalité qui permet de calculer les captures potentielles d'un stock inexploité de mollusques à partir des données de distribution de taille dans la population. Cette technique donne des résultats comparables à ceux obtenus par l'étude de la composition par âges, tant pour le calcul des coefficients de mortalité que pour le calcul des rendements. Elle pourrait donc être un outil particulièrement utile lorsqu'on veut, ou doit, se passer des lectures d'âge systématiques.

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THE estimation of potential yields of an unexploited stock usually requires the knowledge of population structure. For several species, and particularly for mollusca living in sediments, age determination by counting annual growth rings may be difficult because of shell alteration and the uncertainty of this determination for animals older than 8 yr (Newcombe 1935).

Jones (1974) showed that it was possible to use length composition data instead of age composition data to compute exploitation and mortality rates in an exploited population. Such a technique may be useful as length measurements are fast and easy especially when an inventory of a large stock is required or when age readings are impossible. We followed this lead to estimate the potential yield of an unexploited stock of soft-shell clam, *Mya arenaria*.

Method — Two fundamental equations are used to describe the evolution of a population with time. The first one is the growth equation found by von Bertalanffy (1938) to be:

$$(1) \quad L_t = L_\infty \left[1 - e^{-K(t-to)}\right]$$

and the second one is the cohort evolution in number with time, expressed as

$$(2) \quad N_t\, dt = Re^{-Zt}\, dt$$

where $N_t\, dt$ is the number of individuals present in the sea with ages between $t \pm {}^1/_2\, dt$, R is the initial number at $t = 0$, and Z is the instantaneous mortality rate due to all causes (Beverton and Holt 1956). On the basis of those equations, Jones and van Zalinge (1980) suggested that the best equation to calculate the mortality rate Z from length data was

$$(3) \quad \ln \sum_{L} N = \frac{Z}{K} \ln (L_\infty - L) + \ln C$$

where $\sum_{L} N$ is the total number of individuals in the cohort greater than or equal to length L, K and L_∞ are the growth parameters of equation (1), and C is a constant independent of L.

The mortality rate Z and the theoretical values of abundance for each length-class can now be estimated from the regression line described by equation (3). This operation filters out the variations in abundance of successive cohorts in the population and gives an average estimate of potential catches.

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TABLE 1. Abundance of length-classes of a clam population, unexploited ($\hat{N}_L$) and exploited ($\hat{N}_e$), and potential catches first year of exploitation (Y_0) and at equilibrium level (Y_e).[a]

L, mm	$N_L \cdot 10^{-6}$	$\hat{N}_L \cdot 10^{-6}$	$\bar{W}_L$, mg	Δt, year	$Z\Delta t$	Y_o, m.t.	$\hat{N}_e \cdot 10^{-6}$	Y_e, m.t.
18	115.8	59.1	52			—		—
20	86.4	54.8	70			—		—
22	55.0	50.6	92			—		—
24	35.2	46.6	118			—		—
26	28.8	43.8	148			—		—
28	31.3	39.8	184			—		—
30	16.0	36.9	224			—		—
32	11.5	33.2	271			—		—
34	18.6	31.1	323			—		—
36	16.0	27.5	381			—		—
38	15.4	25.3	446			—		—
40	12.8	22.5	519			—		—
42	10.2	20.2	598			—		—
44	16.0	18.2	685			—		—
46	21.8	15.6	781			—		—
48	15.4	13.9	885			—		—
50	19.2	12.0	998	0.86	1.08	7.3	12.0	7.3
52	17.9	10.3	1120	0.93	1.16	6.8	4.1	2.7
54	16.0	8.7	1251	1.00	1.25	6.2	1.3	0.9
56	8.3	7.3	1393	1.09	1.36	5.6	0.4	0.3
58	9.6	6.0	1544	1.19	1.49	4.8	0.1	0.1
60	9.0	4.9	1706	1.32	1.65	4.1	0	—
62	7.0	3.8	1880	1.47	1.84	3.3	0	—
64	3.8	2.9	2064	1.67	2.09	2.5	—	—
66	1.9	2.2	2261	1.92	2.41	1.8	—	—

[a] N_L = observed numbers; $\hat{N}_L$ = calculated numbers from $\ln \sum_L N_L = -1.961 + 3.146 \ln (L_\infty - L)$, ($r = 0.97$); $Z = 1.25$.

When introducing a new fishing mortality rate in the population, the number of animals whose size is greater than the length at first capture (L_c) will change. Knowing the number of animals at the first capture (R_{Lc}) from equation (3), and the new mortality rate (Z'), it is possible to calculate the length composition at equilibrium level as

$$(4) \quad N_L\, dL = R_{Lc}\, e^{-Z'\delta t}\, dL$$

where N_L is the number of individuals present in the sea with length between $L \pm dL$, and δt is the interval of time necessary to grow from the length at the first capture (L_c) to L. δt can be calculated from equation (1) as

$$\delta t = \frac{1}{K} \ln \left(\frac{L_\infty - Lc}{L_\infty - L}\right).$$

The potential catches for each length-group can then be computed from standard equations (Beverton and Holt 1957) as

$$(5) \quad C_{L_1/L_2} = \frac{F}{Z\Delta t} N_{L_1} (1 - e^{-Z\Delta t})$$

$$(6) \quad Y_{L_1/L_2} = \frac{F}{Z\Delta t} N_{L_1} \cdot \bar{W}_{L_1/L_2} (1 - e^{-Z\Delta t})$$

in number and weight, respectively, where F is the instantaneous mortality rate due to fishing and Δt is the time necessary to grow from L_1 to L_2. Δt depends on the selected lengths interval and, as previously, it can be described as

$$(7) \quad \Delta t = \frac{1}{K} \ln \left(\frac{L_\infty - L_1}{L_\infty - L_2}\right).$$

$\bar{W}_{L_1/L_2}$ is the average weight of individuals having a length between L_1 and L_2; this average may be considered equivalent to the calculated weight, from a length–weight relationship, for the middle point of a particular length-class.

The basic assumptions for the application of this method are a constant mortality rate with age and individual growth curves following von Bertalanffy curves with same values of K and L_∞.

Application to a clam population — During the summer of 1978, part of the *M. arenaria* stock of Rimouski (Québec) was studied. This is a coastal zone where mollusc exploitation is forbidden.

A total of 1750 clams were dug out from 60 stations distributed on a 2.4-km^2 area. For each individual, dry flesh weight was measured and age was determined by counting annual growth rings. The ratio of the total wet weight to the dry flesh weight was found to be 24/1. Von Bertalanffy growth parameters were calculated from the Ford–Walford method and were found to be:

$L_\infty = 80$ mm
$K = 0.08$
$t_o = 0.19$ yr

The length–dry weight relationship was defined as

$$W_L = 0.00758\, L^{3.0} \text{ mg}$$

Lengths were grouped in 2-mm classes and the regression between $\ln \sum_{L} N$ and $\ln (L_\infty - L)$ was computed from (3). As the stock is unexploited total mortality rate (Z) equals the natural mortality rate (M) and the regression gives an estimate for M/K equal to 3.14; thus it follows that M is equal to 0.25. Average abundance for each length-class was then computed with this regression. The data are given in Table 1.

A value of $F = 1.00$ was chosen. This value is not the optimum F given by Beverton–Holt tables but, in fact, any value could be selected for our purpose.

As local fishery regulations specify the length at first capture to be 50 mm, and as constant recruitment was assumed, the value of R_{Lc} is equal to 12.0×10^6 individuals.

Abundance and potential catches at equilibrium level are given by equations (4) and (6), respectively. Total catch is 11.3 t/yr (dry weight) or 271 t/yr (wet weight) (Table 1).

To test the validity of that method and to compare results, an estimate of yields was made using the classical age-composition method. Mean year-class lengths and weights were calculated as well as the regression line:

$$(8) \quad \ln N_t = \ln N_o - Mt$$

The natural mortality rate is found to be equal to 0.23. The calculated year-class abundances are given in Table 2. Assuming a constant recruitment (12 yr and 17.2×10^6 animals), it is easy to compute age composition and corresponding catches until the equilibrium level is reached. Annual yield would be then 13.6 t/yr (dry weight) or 327 t/yr (wet weight) (Table 2).

Transition years — When a fishery begins on a species, or when fishing effort is changing, a certain delay exists between the beginning of exploitation and the achievement of the equilibrium level. We calculated that delay and the possible catches in that period.

These estimates are easy to compute with age composition data. However, with size composition data, the computation becomes more difficult as the time interval needed to grow from L_1 to L_2 is not necessarily 1 yr. One must then calculate the annual total catches instead of length-class catches. Catches are found to be continuously decreasing from the 1st yr of exploitation to the equilibrium level where they stabilize near an asymptotic value Y_e. This evolution can be described by a decreasing exponential function like

$$(9) \quad Y_t = (Y_o - Y_e)\, e^{-ZT} + Y_e$$

when Z is the instantaneous mortality rate, Y_o is the yield during the 1st yr of exploitation, Y_e is the yield when equilibrium level is reached, and T is the year of exploitation (Appendix 1).

Table 3 shows the catches calculated by both methods.

Discussion — The results show that an estimation of mortality rate can be obtained either from age composition data or from length composition data as the calculated values are very similar.

Differences appear, however, in expected catches: the total catches estimated from length composition data are always about 20% less than those calculated with age composition data. This can be explained by the fact that the exploited phase begins somewhere between age 12 and 13 yr. We assumed 12 yr as the age at first capture which means that the length at first capture was 49 mm. Fishermen, in fact, do not discern differences of 1 mm but our model does. An increment of 1 mm in size is equivalent to 5 mo of life during which a significant number of individuals die without being caught. As the total catch is strongly influenced by catches at the beginning of the exploited phase, big variations in total catches could result and, for that matter, results obtained with length composition data should be more realistic as there is no uncertainty in the length at first capture.

Other criticisms could be formulated. In this size composition technique, we assumed continuous individual growth following the von Bertalanffy equation. Most researchers use this equation for mollusca, but several species show either a logistic type growth curve or a "stair" type growth. Utilization of the von Bertalanffy equation appears then as a simplification which may introduce a certain bias in the results.

We also assumed that recruitment was constant, which is not necessarily true. Even if we do not consider natural fluc-

TABLE 2. Abundance of age-classes of a clam population, unexploited ($\hat{N}_t$) and exploited (N_e), and potential catches at equilibrium level (Y_e).[a]

Age	L_t, mm	$\hat{L}_t$, mm	$N_t \cdot 10^{-6}$	$\hat{N}_t \cdot 10^{-6}$	$\bar{W}_t$, mg	$N_e 10^{-6}$	Y_e, m.t.
1	6.6	5.0			1		—
2	11.7	10.8	80.6		10		—
3	16.8	16.1	256.0		32		—
4	20.0	21.0	170.6		70		—
5	23.3	25.6	67.2	91.1	126		—
6	27.5	29.7	37.8	72.2	197		—
7	31.0	33.6	22.4	57.2	286		—
8	34.5	37.2	39.0	45.3	387		—
9	38.2	40.5	29.4	35.9	500		—
10	42.3	43.5	29.4	28.5	619		—
11	46.0	46.3	42.2	22.5	747		—
12	49.0	48.9	30.7	17.9	880	17.9	9.1
13	52.5	51.3	20.3	14.2	1015	5.2	3.1
14	55.5	53.5	12.8	11.2	1151	1.5	1.0
15	54.0	55.5	14.1	8.9	1286	0.4	0.3
16	58.5	57.4	9.0	7.1	1422	0.1	0.1
17	63.4	59.1	2.6	5.6	1552	0	0
18	59.3	60.8	3.2	4.4	1690	0	0

[a] $\hat{L}_t$ = length calculated from von Bertalanffy equation, $\hat{N}_t$ calculated from $\ln \hat{N}_t = 10.28 - 0.23\, t$ ($r = 0.91$).

TABLE 3. Estimation of catches for the transition period (m.t.) with $Z = 1.25$.

	Y_o	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6	Y_e
Age composition	53.2	25.4	17.0	14.6	13.9	13.7	13.6	13.6
Length composition	42.5	20.2	13.8	12.0	11.5	11.3	11.3	11.3

tuations, the rate of recruitment estimated from the unexploited population will not likely be valid for the exploited population, especially if the rate of recruitment is dependent on the abundance of the adult exploited stock.

Estimation of potential catches from age composition data is considered to be the better method because it can take into account fluctuations in recruitment. On the other hand the size composition method avoids age reading errors which may be important in the fishery in this case, since age at first capture varies between 6 and 12 yr (Lamoureux 1977). The latter method also permits integration over every observed size group of the area and it is interesting to note that the choice of any length-class interval has no importance for the application of the technique.

The age composition method remains a powerful technique, but age determination is a time-consuming operation and precision decreases with animal age. In theory, the utilization of length composition data is less satisfying since, in fact, length is not directly proportional to age, but in practice, it remains an easy and fast technique which can be very useful in the inventories of large stocks.

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Appendix 1 — To derive the equation of diminution of total catches following successive years of exploitation, we must refer to the evolution of total population present in the sea year after year, as the catches in number or weight are directly proportional to the size of this population. We use:

N_1: Number of animals at the recruitment, assumed constant;
M: instantaneous mortality rate due to natural causes;
F: instantaneous mortality rate due to fishery;
$Z = M + F$: instantaneous mortality rate due to all causes;
t: years of exploitation,
$t = o$: equilibrium level before exploitation,
$t = e$: new equilibrium level with exploitation;
n: maximum theoretical age that can be reached by any cohort.

At any equilibrium level, every individual is issued directly from recruitment N_1 and subsequently the number of individuals of each age can be expressed as, for the first equilibrium level (only natural mortality):

$$N_1 \text{ (age I)}, N_1e^{-M} \text{ (age II)}, N_1e^{-2M} \text{ (age III)}, \ldots,$$

$$N_1e^{-(n-2)M} \text{ (age } n-1), N_1e^{-(n-1)M} \text{ (age } n).$$

Total number of individuals present in the sea at that time is then:

$$NT_o = N_1 \sum_{a=0}^{a=n-1} e^{-aM}$$

which is equivalent to

$$(1) \quad NT_o = N_1 \left(\frac{1 - e^{-nM}}{1 - e^{-M}}\right).$$

The same calculation can be made for the new equilibrium level created by the fishery:

$$NT_e = N_1 \sum_{a=o}^{a=n-1} e^{-a(M+F)}$$

and

$$(2) \quad NT_e = N_1 \left[\frac{1 - e^{-n(M+F)}}{1 - e^{-(M+F)}}\right].$$

For the intermediate years, between $t=o$ and $t=e$, we have the following situations.

$$t = 1: N_1, N_1e^{-(M+F)}, N_1e^{-(2M+F)}, N_1e^{-(3M+F)}, \ldots, N_1e^{-[(n-1)M+F]}$$

$$t = 2: N_1, N_1e^{-(M+F)}, N_1e^{-(2M+2F)}, N_1e^{-(3M+2F)}, \ldots, N_1e^{-[(n-1)M+2F]}$$

$$t = (e-1): N_1, N_1e^{-(M+F)}, N_1e^{-(2M+2F)}, N_1e^{-(3M+3F)}, \ldots, N_1e^{-[(n-1)M+(n-1)F]}.$$

Then, for each year t, total population present in the sea can be expressed as:

$$(3) \quad NT_t = N_1e^{-t(M+F)}\left[\frac{1 - e^{-(n-t)M}}{1 - e^{-M}}\right] + N_1\left[\frac{1 - e^{-t(M+F)}}{1 - e^{-(M+F)}}\right]$$

and this last relation gives the diminution of population size in the time interval from $t = o$ to $t = e$.

n is a theoretical age which may be considered as a large number, that simplifies equation (3) to

$$(4) \quad NT_t = \left(\frac{N_1}{1 - e^{-M}}\right) e^{-tZ} + \left(\frac{N_1}{1 - e^{-Z}}\right)(1 - e^{-tZ})$$

If we admit the same simplication for equations (1) and (2), after rearrangement equation (4) becomes

$$(5) \quad NT_t = (NT_o - NT_e)\, e^{-tZ} + NT_e.$$

LETTERS AND COMMENTS/LETTRES ET COMMENTAIRES

Size of Exploited Lake Whitefish

COMMENT

Healey (1980) noted that cannibalism had been suggested an "important population regulating" mechanism in northern lakes, but he did not deal specifically with cannibalism in his discussion of the influence of exploitation on lake whitefish, perhaps because "there is no evidence of intraspecific predation" (Power 1978). I suggest the results he observed, i.e. the increased growth and recruitment, occurred through removal by his fishery of adults which cannibalize young in a predation selective for large size, at least up to some limiting size (Forney 1976). Ivlev (1961) showed the median size of a prey population is moved toward smaller size by a predation for large size. Reduction of the intensity of the cannibalism by removal of adults would move the median to larger size; as Healey observed the "average size at age increased after exploitation," from which he inferred an increased rate of growth. Exploitation has been related to larger size, and to rapid growth also, in other populations of lake whitefish (e.g. Mills and Beamish 1980). Regulation of fish populations through cannibalism is likely (Chevalier 1973; Fox 1975), particularly in lake whitefish, whose food is governed less by preference than by availability (Scott and Crossman 1973). — F. G. BARBER, *Department of Fisheries and Oceans, Ocean and Aquatic Science Affairs Branch, Ottawa, Ont. K1A 0E6.*

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REPLY

Barber suggests that reduced cannibalism could account for increased size and recruitment in exploited lake whitefish populations (Healey 1980); however, he does not indicate the conditions under which cannibalism would, in his view, be a sufficient explanation for my results and those of others (Healey 1975). For cannibalism to cause poor recruitment and small size, I believe three conditions must be satisfied: cannibalism must occur, it must be directed at appropriate size- and age-classes in the population, and it must occur at a rate sufficient to regulate size and recruitment. I shall consider whether these conditions are satisfied by the available evidence.

Large whitefish are certainly capable of feeding on small fish, and fish (mainly ninespine stickleback, *Pungitius pungitius*, and slimy sculpin, *Cottus cognatus*, occured in about 8% of whitefish stomachs from my lakes (M. C. Healey unpublished data). Juvenile whitefish, however, were not identified in any of 800 whitefish stomachs examined between 1971 and 1974. The evidence does not, therefore, satisfy the first condition. The hypothesis is not disproved, however, as cannibalism may have occurred at a time for which I had no samples, or at such a low rate that the chance of detecting it with a sample of 800 stomachs was small. When, and at what rate, cannibalism would have to occur to account for my results must also be considered.

In Drygeese, the most heavily exploited of my lakes, greatest recruitment to the fishable population was of fish that were in their first and second summers of life at the time exploitation began. Fish of most age-classes increased in size after exploitation. To account for the observed pattern of recruitment in Drygeese, cannibalism would have to be most intense on fish in their first and second summers, but to account for the increases in size, cannibalism would have to affect virtually all ages. While they do feed on fish, lake whitefish have a small subterminal mouth and are not well adapted to such feeding. Fish found in the stomach samples from my lakes did not exceed 50 mm total length. Young whitefish grow rapidly during their 1st yr of life, reaching 50–60 mm by July of their first summer and 100–110 mm by September (Hart 1931; Bidgood 1973; K. Dietz, Alberta Dep. of Energy and Natural Resources, 1980, personal communication). While lake whitefish adults might easily cannibalize eggs, larvae, and early postlarval whitefish, it seems unlikely that they would be efficient at cannibalizing young whitefish after July of their first summer.The largest whitefish, 50–55 cm long, might be physically capable of ingesting young whitefish in their second summer, which are 12–18 cm long, but it is inconceivable that any older age-classes could be cannibalized. The sizes and ages of young whitefish that older whitefish might potentially cannibalize, therefore, are different from the sizes and ages of whitefish which survived better and increased in size after exploitation.

The second necessary condition under which cannibalism might account for my results, therefore, is not satisfied.

Failure to satisfy two of the three necessary conditions under which cannibalism might account for my results is probably sufficient grounds for rejecting the hypothesis. The minimum rate of cannibalism necessary to regulate recruitment, however, suggests that the hypothesis cannot be categorically rejected as an explanation for increased recruitment. Assuming that regulation of recruitment occurs between June of the whitefish's 1st yr of life, and September of their 2nd yr, as would be required to explain the results from Drygeese Lake (Healey 1980), then cannibalism could be spread over 480 d or more. The adult population in unexploited Drygeese Lake was on the order of 4000 fish, and about 600–800 fish were aged 6+, the usual age of sexual maturation. At least 600–800 fish must recruit to age 2+, therefore, to provide for 600–800 at age 6+. After removal of the adult whitefish, recruitment to age 2+ in Drygeese increased 3–4 times. Thus, minimum recruitment after removal of the adults was 1800–3600, and minimum cannibalism to regulate recruitment involves 1200–2400 juveniles. With 4000 adults in the population, the rate of cannibalism required to remove this many juveniles is only 6×10^{-4} to 12×10^{-4} juveniles eaten per adult per day. These minimum values are unlikely to prevail, as juvenile whitefish die from causes other than cannibalism, and 600–800 age 2+ recruits would not produce a similar number of age 6+ recruits. Nevertheless, these minimum rates do demonstrate the limitations to drawing inferences about cannibalism from a small number of stomach analyses. Even if the rate of cannibalism were 2–3 times the minimum values, 300–500 adult stomachs would have to be examined to find even one cannibalized juvenile.

In summary, it is my view that the available evidence fails to satisfy the necessary conditions under which reduced cannibalism would be a reasonable explanation of increased size and recruitment in exploited whitefish populations. Because of the very low rates of cannibalism that could affect recruitment, however, it is not possible, with present evidence, completely to reject cannibalism as a mechanism contributing to control of recruitment at the youngest age-classes. — M. C. HEALEY, *Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, B.C. V9R 5K6.*

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BOOK REVIEWS/COMPTES RENDUS

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The Assessment of Sublethal Effects of Pollutants in the Sea. *A Royal Society Discussion edited by H. A. Cole. The Royal Society, London, U.K. 1979. viii + 235 p. £19.10.*

Research on the sublethal effects of marine pollutants accelerated dramatically in the 1970s. Much of this science dealt with single organisms exposed to toxicants under controlled laboratory conditions. Interpreting, predicting, and controlling serious pollution problems in the sea by directly applying laboratory data and conclusions is often difficult or impossible. This is the principal theme of the book, which is the proceedings of a Royal Society Discussion Meeting held in London in May 1978. The principal objectives of the meeting were to discuss the "state-of-the-art" of assessing sublethal effects of marine toxicants and to describe procedures and approaches for linking laboratory-derived, effect–concentration relationships with measures of biologically significant pollutant effects in field populations and communities. The resulting book is unique because it contains comprehensive, critical, and very readable review papers prepared by experienced marine pollution scientists. The book covers a wide range of topics, including sublethal bioassays, physicochemical fate of pollutants, bioaccumulation, monitoring procedures, experimental ecosystems, and British regulatory approaches.

A number of ideas and principles important to current marine pollution research are expressed among the 14 chapters of the book. For bioassays, it is important to relate experimental design in sublethal tests, such as the length of the test exposure, to the organism's lifespan. Behavioral bioassay techniques, because of the sensitivity and multifunctions of behavior, should be more frequently used in effluent and water quality biomonitoring activities. On the chemistry of pollutants in the sea, such as metals or hydrocarbons, the material's chemical form in the aqueous environment needs to be thoroughly understood because of its effect on bioavailability and toxic effects. Both physicochemical and biological measurements are needed together for assessing water quality in coastal areas.

Techniques and problems of marine biomonitoring are well covered in the book and some interesting points are made. Waldichuk believes that the apparent absence of recorded sublethal effects to individual organisms in the sea may be due to the "sublethal effect" becoming lethal in the sea, and hence the organisms are no longer present to monitor. Bryan makes the seemingly obvious but overlooked point that organisms that can regulate heavy metals are unsuitable for analysis as indicators of the availability of metals in seawater. Promising monitoring tools under development are described and include an examination of skeletal abnormalities in fish caught at sea, and the use of "scope for growth" measurements, and cytochemical and biochemical methods, for measuring pollutant stress in bivalves, organisms being given considerable attention in current monitoring programs.

Work conducted on organisms in experimental enclosures is reviewed in two excellent chapters. The main value of such enclosures is that they provide the important opportunity to study the movement of pollutants from pelagic to benthic organisms, and to calibrate and test techniques for monitoring pollutant effects. Because of the variability of field populations and communities, the work points out the difficulties of evolving simple generalizations about pollutant stress within marine ecosystems. Some novel concepts about the response of field populations and communities to pollutants are then presented. Organisms may change their life history strategies when under pollutant stress; such adaptation could be more important than tolerance as a mechanism for coping with pollution. The hypothesis is also presented by Cushing that fish populations may be more vulnerable to pollutants when they are being overfished or seriously affected by climatic change.

The book ends, with two chapters by Preston and Cole, on the theme that it began with – that there is the pressing need for a link between laboratory and field studies and evidence on the extent to which fish and invertebrate populations in the sea are significantly affected or reduced by pollution. This theme is valid but in this reviewer's opinion should not negate the continued production of laboratory data on toxic effects and strong statements about possible effects in nature — it is better to err on the side of caution.

The book has been carefully produced and contains few errors in content or typing. However, the book has some limitations. It is organized the same way as the meeting — some reorganization of chapters, such as putting the bioassay, monitoring, and ecosystem papers in separate sections, would have improved the presentation to the reader. The discussions take up valuable space and might have been condensed for easier grasp of the main questions and points of controversy. Some terms, such as stress and synergism, should have been standardized among chapters. Some unfortunate technical limitations include the very confusing double-numbering of the pages, and the absence of an index, which would have been most useful. The book is also very expensive, which makes it a major purchase for private libraries and may make

the libraries content with the previous publication (Phil. Trans. R. Soc. Lond. 284 (B): 397–633). These problems notwithstanding, I recommend the book as essential reading and a reference source for all active marine researchers, regulators, and managers. For some years, it will be a key reference on the sublethal effects of marine pollution, and as such is an important contribution to the field.

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COMING NEXT MONTH / À PARAÎTRE LE MOIS PROCHAIN

Acute toxicity of nitrite to rainbow trout (*Salmo gairdneri*): effects of pH, nitrite species, and anion species **R. C. Russo, R. V. Thurston, and K. Emerson**

Estimating growth parameters for Pacific halibut from mark–recapture data **D. A. McCaughran**

Distribution of zooplankton populations within and adjacent to a thermal plume **M. S. Evans**

Oxygen requirements for growth of young coho (*Oncorhynchus kisutch*) and sockeye (*O. nerka*) salmon at 15°C **J. R. Brett and J. M. Blackburn**

Distribution of eggs by depth and vegetation type in Pacific herring (*Clupea harengus pallasi*) spawnings in southern British Columbia **C. W. Haegele, R. D. Humphreys, and A. S. Hourston**

Determination of sexual maturation stages of Atlantic salmon (*Salmo salar*) captured at sea **D. R. Idler, S. J. Hwang, L. W. Crim, and D. Reddin**

Prediction of total phosphorus concentrations, chlorophyll *a*, and Secchi depths in natural and artificial lakes **D. E. Canfield Jr., and R. W. Bachmann**

Application of a multispecies approach for assessing the population abundance and the age structure of fish stocks **J. Majkowski**

Reproductive behavior of hybrid charr (*Salvelinus fontinalis* × *S. namaycush*) **A. H. Berst, A. R. Emery, and G. R. Spangler**

Stream periphyton development in relation to current velocity and nutrients **R. R. Horner and E. B. Welch**

Notes

Extraction of living meiofauna from marine sediments by centrifugation in a silica–sol–sorbitol mixture **P. Schwinghamer**

Effects of streamflow and upwelling on yield of wild coho salmon (*Oncorhynchus kisutch*) in Oregon **D. L. Scarnecchia**

Immunoenzyme microscopy for differentiating among systemic bacterial pathogens of fish **D. H. Lewis**

Empirical relationships between phytoplankton and zooplankton biomass in lakes **E. McCauley and J. Kalff**

Capturing fish measurements and counts with calipers and probe interfaced with a computer or pocket calculator **D. E. McAllister and R. J. Planck**

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Volume 38, No. 3, March 1981 Volume 38, n° 3, mars 1981

Feeding ecology of capelin (*Mallotus villosus*) in the estuary and western Gulf of St. Lawrence and its multispecies implications
J-P. Vesin, W. C. Leggett, and K. W. Able 257–267

Influence of drilling muds on the primary chemosensory neurons in walking legs of the lobster, *Homarus americanus* **C. D. Derby and J. Atema** 268–274

Predatory feeding behavior of *Epischura lacustris* (Copepoda, Calanoida) and prey defense
C. K. Wong 275–279

Equilibrium model of fate of microcontaminants in diverse aquatic food chains
R. V. Thomann 280–296

Restricted least-squares estimates of age composition from length composition
W. G. Clark 297–307

Evidence of an adaptive basis for geographic variation in body morphology and time of downstream migration of juvenile Atlantic salmon (*Salmo salar*)
B. E. Riddell and W. C. Leggett 308–320

Evidence of adaptive polygenic variation between two populations of Atlantic salmon (*Salmo salar*) native to tributaries of the S. W. Miramichi River, N.B.
B. E. Riddell, W. C. Leggett, and R. L. Saunders 321–333

A model for responses of vertebral numbers in fish to environmental influences during development **C. C. Lindsey and A. N. Arnason** 334–347

Differential response to water current by two homozygous LDH phenotypes of young rainbow trout (*Salmo gairdneri*) **T. G. Northcote and B. W. Kelso** 348–352

Shifts in aquatic insect populations in a first-order southern Appalachian stream following a decade of old field succession **J. D. Haefner and J. B. Wallace** 353–359

Notes

Northern squawfish (*Ptychocheilus oregonensis*) predation on juvenile salmonids in sections of the Willamette River basin, Oregon
D. V. Buchanan, R. M. Hooton, and J. R. Morin 360–364

Drug immobilization of walrus (*Odobenus rosmarus*)
D. P. DeMaster, J. B. Faro, J. A. Estes, J. Taggart, and C. Zabel 365–367

Use of beef tallow as an energy source in coho salmon (*Oncorhynchus kisutch*) rations
T. C. Yu and R. O. Sinnhuber 367–370

Estimation of potential catches of an unexploited stock of soft-shell clam (*Mya arenaria*) from length composition data **J.-C. F. Brêthes and G. Desrosiers** 371–374

Letters and comments/Lettres et commentaires: Size of exploited lake whitefish: Comment **F. G. Barber**; Reply **M. C. Healey** 375–376

Book reviews/Comptes rendus: The assessment of sublethal effects of pollutants in the sea (H. A. Cole [ed.]) **P. G. Wells** 377–378

Coming next month/À paraître le mois prochain 379

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